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РЕЗЮМЕ

ВЛИЯНИЕ РАЗЛИЧНЫХ ДОЗ ИЗВЕСТИ НА МИКРОБИОЛОГИЧЕСКУЮ ДЕЯТЕЛЬНОСТЬ ПОЧВЫ — В МОДЕЛЬНОМ ОПЫТЕ

Б. ХЕЛЬМЕЦИ, Л. КАРПАТИ

Почвенно-микробиологические исследования проведены в лабораторных условиях на образцах, взятых из созревающих модельных экспериментов. В модельном опыте изучали два почвенных типа (обогащенная глиной бурая лесная почва, луговая почва) трех различных приисков и к образцам были прибавлены 1/2, 1,0 и 2,0 дозы извести с целью нейтрализации величины Y_1. В процессе лабораторных исследований, по методу Похона, определили общее количество бактерий, количество нитрифицирующих, аэробно-аммонифицирующих, связывающих азот и разлагающих целлюлозу бактерий. Полученные результаты были сравнены с данными необработанного известью контроля. На основе проведенных до сих пор серий исследований пришли к следующим заключениям. Деятельность бактерий, относящихся к исследуемым физиологическим группам, в обогащенной глиной бурой почве была самая интенсивная при обработке ее 1/2 дозой извести, количество бактерий в этом варианте было самое большое. У луговой почвы полная (1,0) доза извести вызывала наилучший эффект, вследствие чего, в первую очередь, усилилась деятельность нитрифицирующих и разлагающих аэробных бактерий, то есть, количество этих бактерий увеличилось в значительной мере. Двойная (2,0) доза извести почти во всех случаях давала меньше эффекта по сравнению с 1/2 или 1,0 дозами. На основе проведенных до сих пор исследований видно, что в процессе обработки кислых почв известью, для возбуждения почвенных бактерий, у обогащенной глиной лесной почвы требуется 1/2, а у луговой почвы — полная (1,0) доза извести для успешной нейтрализации величины Y_1.

ШИТОВКИ, УГРОЖАЮЩИЕ ПАРКАМ И АЛЛЕЯМ ГОРОДА БУДАПЕШТА

Ш. БОГНАР, Г. ВИНИШ

В процессе определения зараженности щитовками парков и аллей города Будапешта было обнаружено, что в исследуемых местах 79 видов декоративных деревьев и кустарников заражались от случая к случаю или регулярно 45 видами щитовки. Из 45 видов щитовки фауны Венгрии 5 оказались новыми видами (**Heliococcus bohemicus Sulz., Paroudablis piceae Loew., Pulvinaria oxyacanthae L., Lepidosaphes rubi Thiem.** и **Lepidosaphes crataegi Borch.**) Из опознанных видов 18 (**Gossyparia spuria Mod., Pseudochermes fraxini Kalt., Asterolecanium minus Russ., A. variolosum Ratz., Planchonia arabidis Sign., Parthenolecanium corni Bouc., Sphaerolecanium prunastri Fonsc., Physokermes piceae Schr., Leucaspis pusilla Loew., Anamaspis loewi Colv., Lepidosaphes ulmi L., Lepidosaphes rubi Thiem., Chionaspis salicis L., Unaspis euonymi Comst., Pseudaulacaspis pentagona Targ., Carulaspis visci Schr., Epidiaspis leperii Sign., Quadraspidiotus perniciosus Comst.**) определенно угрожают декоративным деревьям и кустарникам наших парков и аллей. Результаты исследований, проведенных по внутреннему содержанию зараженных декоративных деревьев и кустарников показали, что щитовки отнимают значительное количество воды особенно в фазе вегетации и у растений обнаружено определенное «калийное голодание». Против зимующих форм зимой можно защищаться отмывающим опрыскиванием (3 – 5%-ной эмульсией масла для плодовых деревьев, или разбавленной в пропорции 1 : 5 серной извести), далее, во время роения повторно применёнными производными

органических фосфорных эфиров (0,3%-ный Фосфотион, содержащий в себе действующее вещество малатион, далее, 0,1%-ный Lebaycid 50 EC, принадлежащий к группе фентиона). Достоверной защиты против щитовок, угрожающих декоративным деревьям и кустарникам, можно достигнуть не менее, чем двумя опрыскиваниями.

ИЗУЧЕНИЕ ПАТОГЕННОСТИ ВИДОВ Aspergillus В ЭМБРИОГЕНЕЗЕ КУРИННОГО ЯЙЦА

О. ТОТ, А. ПАЛ, Й. ПАДЬ

В эксперименте 0,1 мл суспензию конидий, полученную путем смывания содержащим 1% Tweent, соляным физиологическим раствором с наклонного агара талломов различных видов Aspergillus привили в воздушные камеры инкубированных мягких куринных яиц. В случае вида A. nidulans, с целью сравнения, изучали и вакуум-инфильтрацию конидиума. На основании экспериментальных данных выявили, что вызванная прививкой инфекция привела к тотальному некрозу в первой трети периода инкубации у видов A. fumigatus, A. flavescens, A. fonseacaues, A. parasiticus, A. flavus, A. nidulans и A. oryzae, а у видов A. amstelodami, A. ochraceus и A. candidus некроз распространился на полный период инкубации у половины зародышев. Клиническая картина, наблюдавшаяся в воздушных камерах, в большей части случаев, была специфична на упомянутые грибы; в то же время проявились некоторые, правда слабые, изменения по различным анатомическим формам (как например, накопление различных красительных веществ и поверхностное распространение их в воздушных камерах, распространение разложенных веществ, окраска и состояние различных анатомических форм, состояние зародыша и. т. д.), по всей вероятности, практически непригодные для различных диагнозов, вызванных различными грибами у яиц, однако они являются очень полезными для экспериментальных целей и дают возможность отличить инфекции, вызванные, напримерр, бактериями. Единственным исключением является A. nidulans, вызвавший слегка красноватую пигментацию в своем симптоме на мембране яичной скорлупы, что таким образом облегчило его распознание среди других бактерий. Пропустив последние виды через вакуум-фильтр, некроз замедлился и тотальный некроз появился только в конце инкубационного периода; при этом пигментационный симптом распространялся по крупным порам и покрыл полностью скорлупную мембрану.

РАСПРЕДЕЛЕНИЕ ИНДОЛ-УКСУСНОЙ КИСЛОТЫ И ЕЕ ТРАНСПОРТ В ЗЕЛЁНЫХ И ЭТИОЛИРОВАННЫХ ПОБЕГАХ ФАСОЛИ

М. ВАРГА, Ж. СЕНДРЁ

Содержание ИУК у зародышевых побегов фасоли, выращенных на свете и в темноте, несмотря на сильное разнообразие по вытянутости стеблей, не отличается в значительной мере. Освещение, таким образом, не влияет на общее количество свободной ИУК, распределение её внутри стебля, однако значительно отличается в зелёных и этиолированных растениях. В этиолированных побегах большая часть общего количества ИУК концентрируется в гипокотилях и только небольшая часть его встречается на месте синтеза, в верхушечной части. В зелёных побегах положение обратное. Интенсивность базопетального транспорта ^{14}C – ИУК, примененного на первичных листьях, как в зелёных, так и в этиолированных интактных побегах, значительно снижается при освещении и возрастает в темноте. В процессе транспорта свет способствует коньюгированию ИУК молекул в иммобильный ИУК аспартат и в ИУК глюкозид. На основе полученных результатов можно сделать вывод, что в зелёных и этиолированных побегах фасоли не содержание ИУК, а степень транспорта базопетальной ИУК пропорциональна удлинению стебля.

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EFFECTS OF VARYING RATES OF LIMING ON THE MICROBIOLOGICAL ACTIVITY OF THE SOIL IN A MODEL EXPERIMENT

By

B. HELMECZI, L. KÁRPÁTI

AGRICULTURAL UNIVERSITY, DEPARTMENT OF SOIL SCIENCE AND MICROBIOLOGY, DEBRECEN

Soil microbiology studies were made on samples taken from model experiments performed under laboratory conditions. The model experiment was set up with two soil types (clay-washed brown forest soil and meadow soil) obtained from three growing sites, and 0.5, 1.0 and 2.0 doses of lime were used to neutralize the y_1 value. In the course of laboratory analyses the total number of bacteria in the soil, and the amounts of nitrifying, aerobic ammonifying, N_2-binding and cellulose decomposing bacteria were determined according to Pochon. The results were evaluated in comparison with the numerical values obtained with pots not treated with lime (control). On the basis of the serial examinations performed so far the following observations were made. In clay-washed brown forest soil the activity of bacteria belonging to the physiological groups examined was stimulated to the greatest extent by the 0.5 dose of lime; the number of bacteria was the highest in this case. On meadow soil the full dose (1.0) of lime proved to be the most favourable; it primarily promoted the activity of the nitrifying and aerobic cellulose decomposing bacteria, and the number of these bacteria grew in the greatest measure. The effect of the double dose (2.0) of lime was less than that obtained with 0.5 or 1.0 doses of lime in almost every case. On the basis of the examinations carried out so far it seems probable that in treating acidic soils with lime, half the amount of lime required to neutralize the y_1 value is efficient from the point of view of stimulating the activity of soil bacteria in clay-washed brown forest soils, while the full dose of lime may be necessary for meadow soils.

Introduction

51% of Hungarian soils have unfavourable properties which require improvement in the interests of efficient farming. Of this 51%, 34% is made up of acidic soils, the rest consisting of alkali and sandy soils. A large proportion of the acidic soils in Hungary is made up of acidic forest soils and meadow soils. The effect of liming on the properties of acidic soils and on the activity of the microorganisms living in the soil has been dealt with by many researchers.

According to BERGLUND (1971), under the influence of liming the structure of the clay in heavy soils becomes stabilized, and large, permanent grains are formed. TISDALE—NELSON (1966) found that besides promoting the growth of plants in some soils, liming increased the amount of phosphorus available to the plants and had a positive effect on the microorganisms in the soil. Nitrification is particularly promoted by liming, as most of the organisms transforming ammonium into nitrate require large quantities of active calcium. The non-symbiotic nitrogen-binding organisms also fix the N_2 content of the atmosphere in larger quantities when the soil is adequately limed.

GRINCHENKO (1975) pointed out that calcium and gypsum increased the biological activity of the soil. According to HIRTE (1970a, b) liming increases the activity of microorganisms, particularly that of bacteria. Under the influence of increased microorganism activity organic matter undergoes a more intensive transformation. Soils with higher pH values have a stimulative effect on the bacteria, so that if a sufficient amount of organic matter is present the number of germs in them may increase by 1—2 places of decimals. A similar statement was made in papers by MIRCHINK—ARFENINA (1974) and GANYUSHINA—POPOVA (1975). According to their investigations liming increases the total number of bacteria and reduces the amount of fungi. Repeated treatments with lime increase the ammonifying and nitrifying activities as well.

The question is, however, what quantity of lime will result in a substantial improvement in soil properties, but still be economical. In a four-year experiment carried out under poor precipitation conditions with a soil KCl pH of 4.3 MELNICHUK—POPOVA (1973) found that the grass yield increased to the greatest extent when the soil was treated with dolomite powder, which neutralized fifty per cent of the hydrolytic acidity of the soil. The highest crude protein surplus was also ensured by half a dose of lime combined with NPK fertilization.

In the experiments of BOGDANOV—HLISTOVSKY—OPIMAH (1975), in which 18—20 year after-effects were taken into consideration, the full dose of lime, calculated on the basis of the hydrolytic acidity, gave the best results.

According to TISDALE—NELSON (1966) lime is one of the most important production investments in the farm management system. Its positive effect on the availability of phosphate and microelements, on nitrification, nitrogen binding and on the structure of the soil influences plant production in many ways.

Material and Method

On setting up the experiment our aim was to obtain reliable data on how different rates of liming would influence the living organisms of the soil in two soil types: in a clay-washed brown forest soil and in a typical meadow soil, and further, to find out the effect of liming on the total number of bacteria, and on the number of nitrifying, aerobic protein-decomposing, N_2-binding and cellulose decomposing bacteria.

Our investigations are designed to provide data towards settling the question of whether to apply half or full (possibly even larger) doses of lime.

The analyses were made on incubated soils; plastic cylinders were used for setting up the experiment.

The prepared soils were treated with different quantities of lime powder containing 92.6% $CaCO_3$ as meliorant. In order to neutralize the y_1 value half the calculated dose of lime (1/2) was added to the soil in treatment 1 and a full dose (1.0) in treatment 2; as a control, series not treated with lime were also set up (∅) for both soil types. The experiment was carried out in five replications. Incubation took place in an incubator adjusted to a steady 20°C temperature. In order to ensure optimum moisture content the samples were moistened before ncubation to an extent corresponding to half of Arany's viscosity index, according to Várallyay.

In the first phase of our investigations samples were taken on four occasions, at exactly four week intervals, from each of the two soil types in the experiment: a clay-washed brown forest soil from Kenyeri ($K_A = 28$), and a heavier meadow soil from Hosszúhát ($K_A = 46$).

The samples were taken under sterile conditions, over an alcohol flame, with a hand borer, and then homogenized. The examinations were performed after POCHON—TARDIEUX (1972); the total number of bacteria, and the number of nitrifying, aerobic ammonifying, N_2-binding and cellulose-decomposing bacteria in the samples were regularly determined.

Since different results were obtained for the two soil types, it seemed reasonable to examine the effect of a larger dose, an amount of lime twice as much as that generally used to neutralize the y_1 value (treatment 3; 2.0) on a third sample, that is, on another clay-washed brown forest soil.

Thus samples were subsequently collected from the previous two soil types (a clay-washed brown forest soil from Kenyeri, and a meadow soil from Hosszúhát), as well as from a clay-washed brown forest soil obtained from Nagykanizsa ($K_A = 35$), which were each examined in four treatments: control; 1/2 lime; 1.0 lime and 2.0 lime.

Results

A predetermined number of cells from samples taken simultaneously on four occasions were averaged by treatments and physiological groups, and the logarithm values of the number of bacteria were plotted. Fig. 1 shows the quantitative change in the total bacterium number in clay-washed brown forest soil and typical meadow soil.

In a clay-washed brown forest soil the total number of bacteria was highest in the treatment given a 1/2 dose of lime, while in the case of meadow soil the highest value was obtained in the control; 1/2 dose of lime slightly decreased, and 1.0 lime increased the number of bacteria. In Fig. 2 the number of aerobic ammonifying bacteria is seen on the fourth occasion of sampling. Maximum mean values were obtained with the 1/2 dose of lime in both soil types; the course of the two curves was similar. In Fig. 3 the quantitative change in the aerobic N_2-binding bacteria as a response to liming can be observed. If the averages are considered, in a clay-washed brown forest soil the highest value was obtained with the 1.0 dose of lime, while in the meadow soil the treatments decreased the quantity of N_2-binding bacteria. According to Fig. 4 in the clay-washed brown forest soil the largest number of nitrifying bacteria was obtained with the 1/2 dose of lime; in the meadow soil, by contrast, the 1/2 dose of lime decreased the number of bacteria, and the maximum value was obtained with the 1.0 dose of lime. Fig. 5 shows the quantitative change in the aerobic cellulose-decomposing bacteria.

In clay-washed brown forest soil the number of cellulose-decomposing bacteria stagnated at nearly the same level after liming, while in meadow soil liming resulted in a steady increase in their number.

In our later examinations for reasons already given, two forest soils and a meadow soil were used. The results of these examinations are summarized in Table 1. The logarithmic values of the bacterium numbers are plotted in Figs 6, 7 and 8. Fig. 6 shows the effect of increasing rates of liming on different

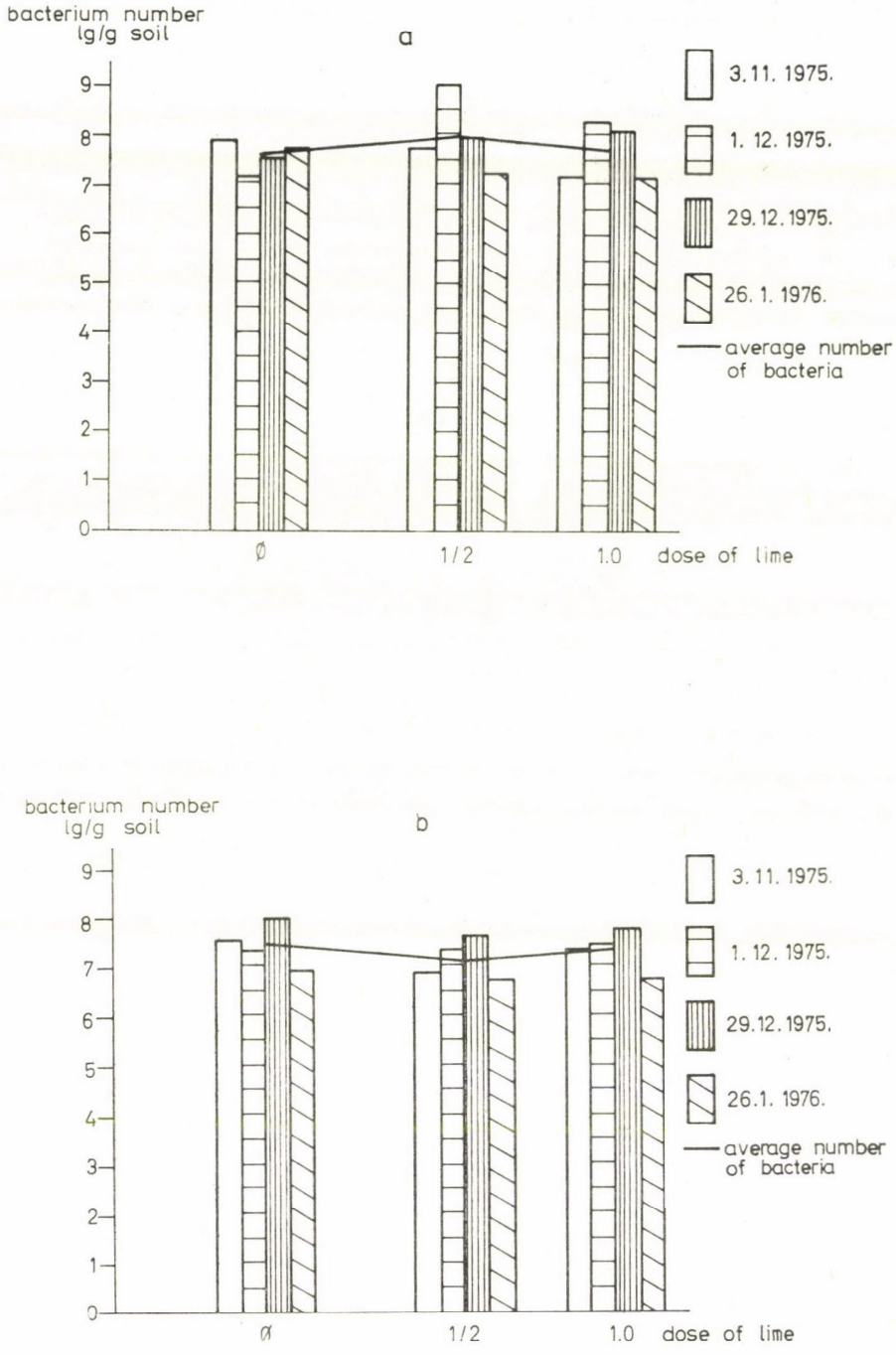


Fig. 1. Changes in the total number of bacteria in the soil (pot experiment); *a)* clay-washed brown forest soil, Kenyeri; *b)* meadow soil, Hosszúhát

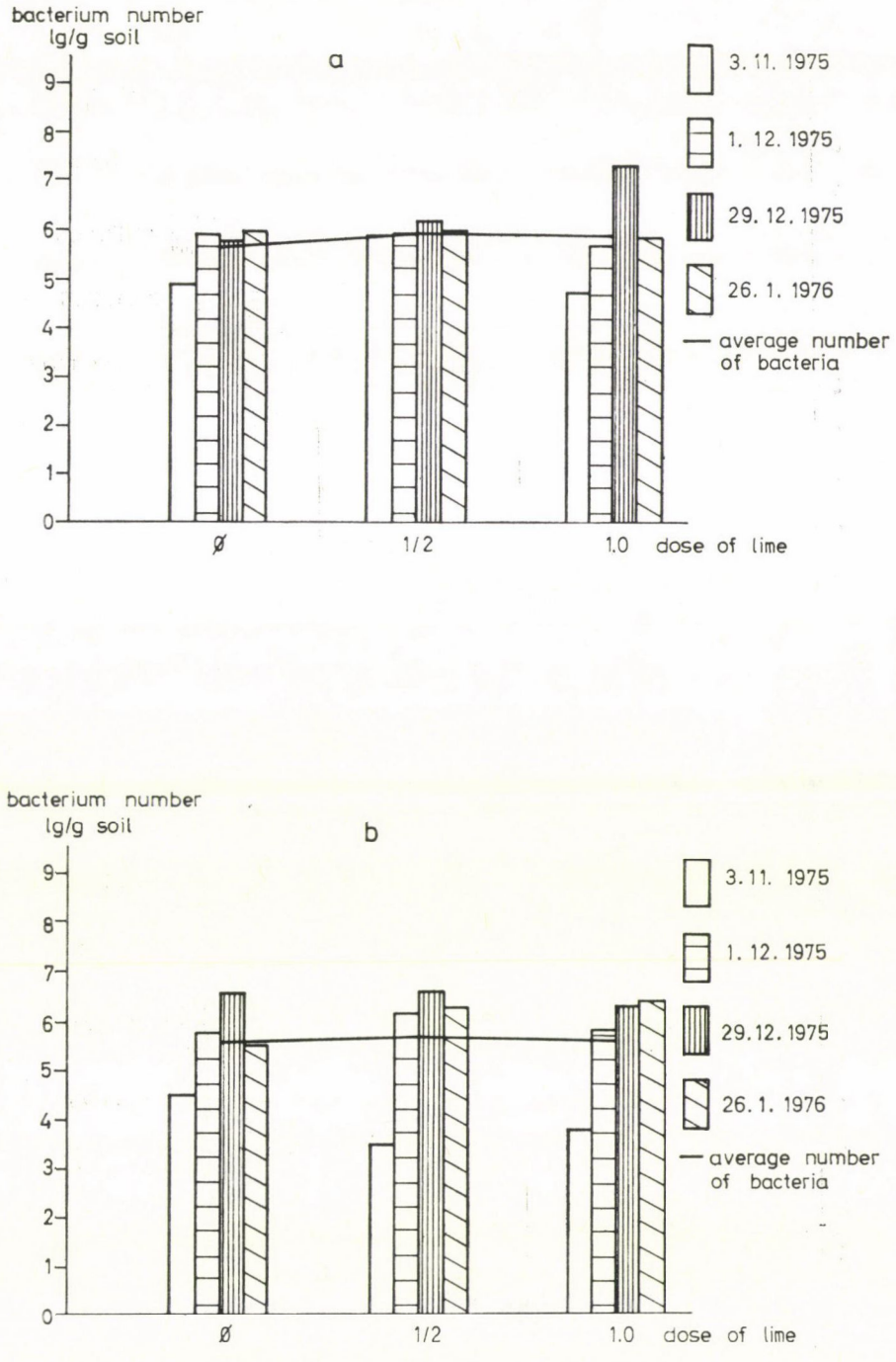


Fig. 2. Number of aerobic ammonifying bacteria in the soil (pot experiment); *a)* clay-washed brown forest soil, Kenyeri; *b)* meadow soil, Hosszúhát

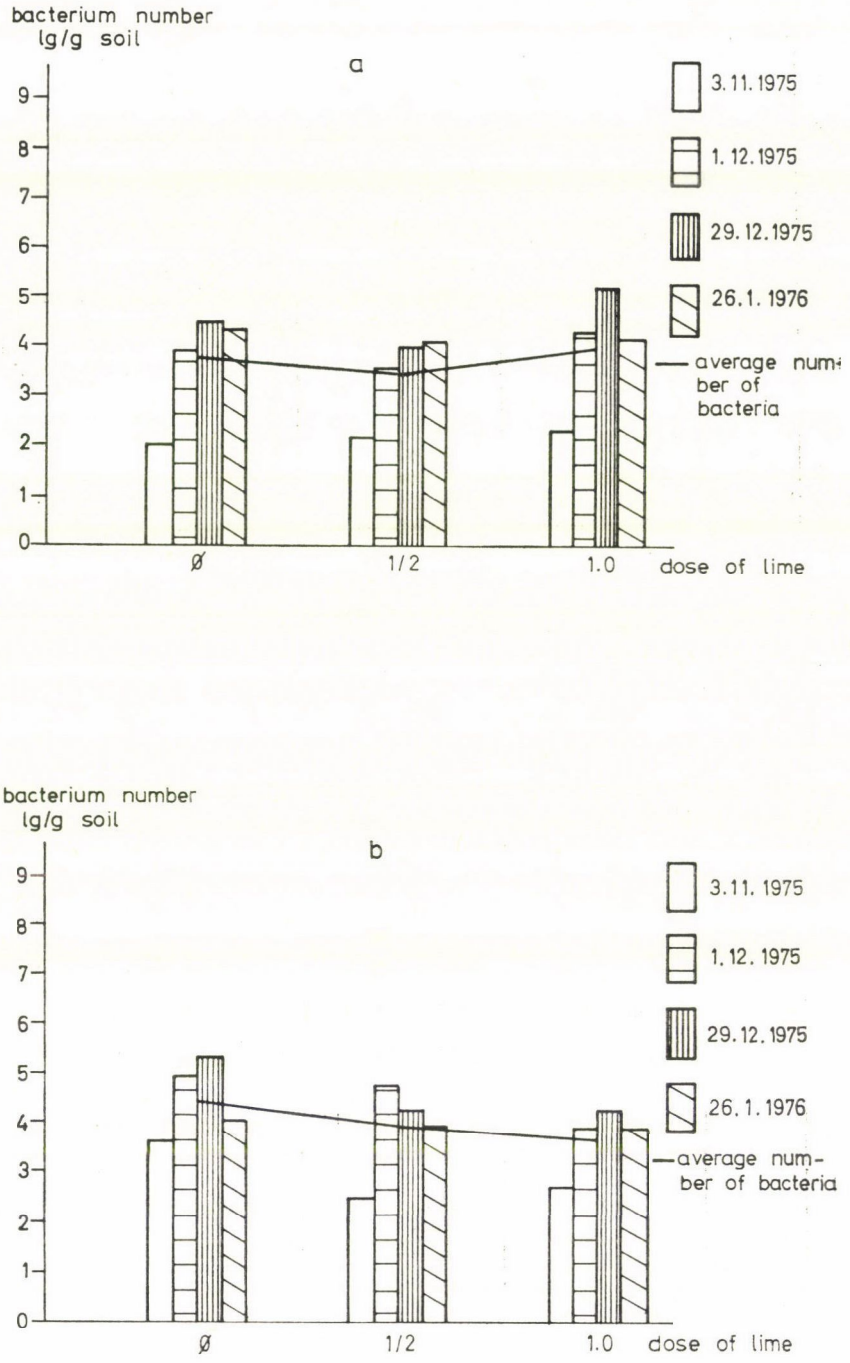
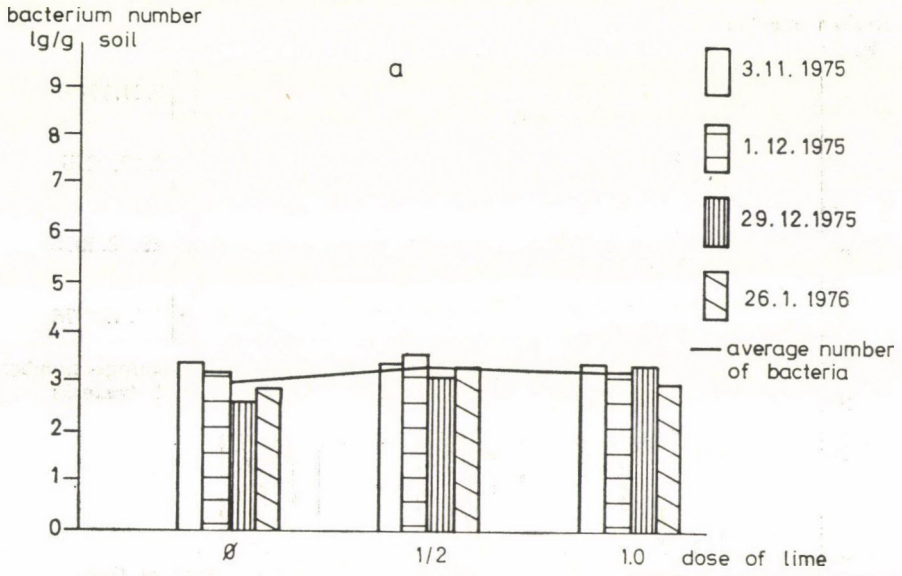


Fig. 3. Number of aerobic N_2-binding bacteria in the soil (pot experiment); *a)* clay-washed brown forest soil, Kenyeri; *b)* meadow soil, Hosszúhát



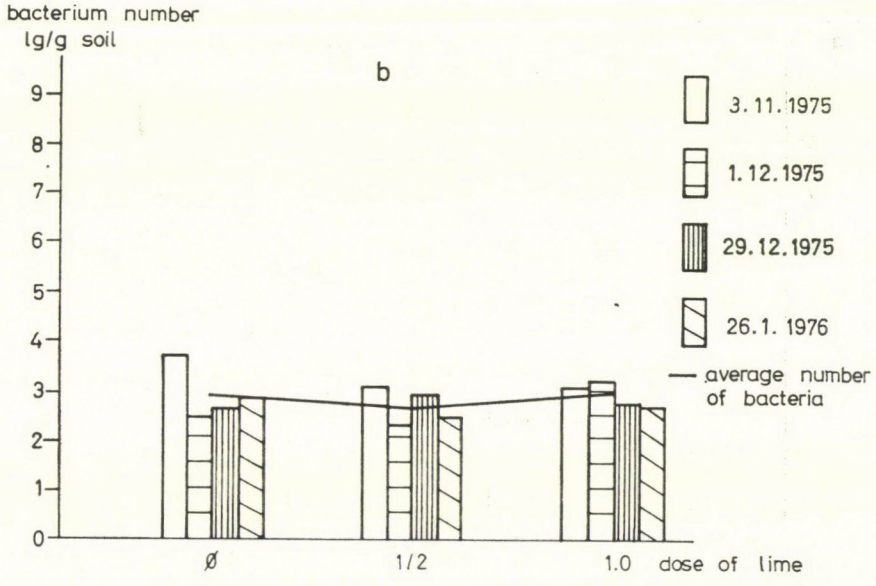


Fig. 4. Number of nitrifying bacteria in the soil (pot experiment); *a)* clay-washed brown forest soil, Kenyeri; *b)* meadow soil, Hosszúhát

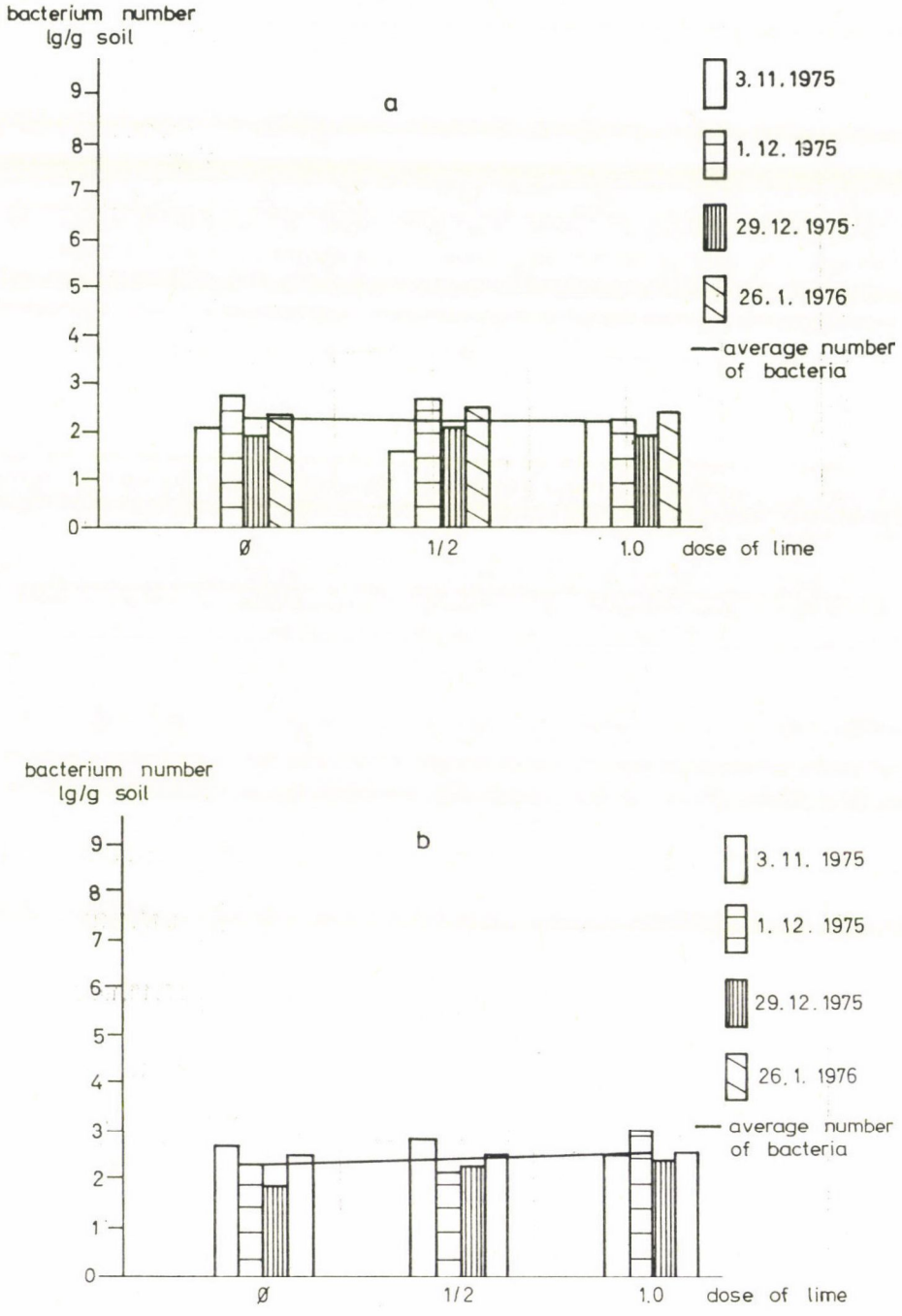


Fig. 5. Number of aerobic cellulose-decomposing bacteria in the soil (pot experiment); *a)* clay-washed brown forest soil, Kenyeri; *b)* meadow soil, Hosszúhát

Table 1

Changes in the number of bacteria belonging to different physiological groups in various soil types as a response to liming

Treatments	Total number of bacteria, 10^6/g soil	Number of nitrifying bacteria, 10^3/g soil	Number of N_2-binding bacteria, 10^3/g soil	Number of aerobic cellulose decomposing bacteria, 10^3/g soil	Number of aerobic ammonifying bacteria, 10^3/g soil
	Meadow soil (Hosszúhát)				
Control	98.3	1.0	57.2	4.4	435.4
1/2 dose of lime	118.4	1.6	27.4	114.7	299.1
1.0 dose of lime	167.2	0.9	180.0	1.7	514.5
2.0 dose of lime	45.4	0.6	28.5	23.3	142.6
	Clay-washed brown forest soil (Nagykanizsa)				
Control	25.1	0.5	16.0	0.2	90.1
1/2 dose of lime	163.1	2.0	19.8	0.2	291.2
1.0 dose of lime	625.3	2.8	56.7	1.3	162.1
2.0 dose of lime	12.5	0.3	19.3	0.3	147.5
	Clay-washed brown forest soil (Kenyeri)				
Control	16.9	0.1	2.7	0.4	25.9
1/2 dose of lime	24.7	0.9	19.1	0.2	123.7
1.0 dose of lime	37.2	0.4	1.6	0.3	1240.9
2.0 dose of lime	3.9	0.6	1.5	0.4	396.5

physiological groups of bacteria found in the incubated sample of soil obtained from Kenyeri.

In this soil the total number of bacteria and the number of aerobic ammonifying bacteria were at a maximum when the soil was treated with the 1.0 dose of lime. The numbers of aerobic N_2-binding and nitrifying bacteria were the highest when the 1/2 dose of lime was applied. The number of aerobic cellulose-decomposing bacteria remained more or less unchanged after liming. Fig. 7 presents the results obtained during the examination of grown forest soil from Nagykanizsa. It can be seen from the figure that maximum values for the total number of bacteria and the number of aerobic N_2-binding, nitrifying and aerobic cellulose-decomposing bacteria were obtained with the 1.0 dose of lime. In the case of the ammonifying bacteria, liming with the 1/2 dose gave the highest bacterium number. In Fig. 8 the results of analyses performed on the typical meadow soil from Hosszúhát are seen. The total number of bacteria and the number of aerobic ammonifying and N_2-binding bacteria

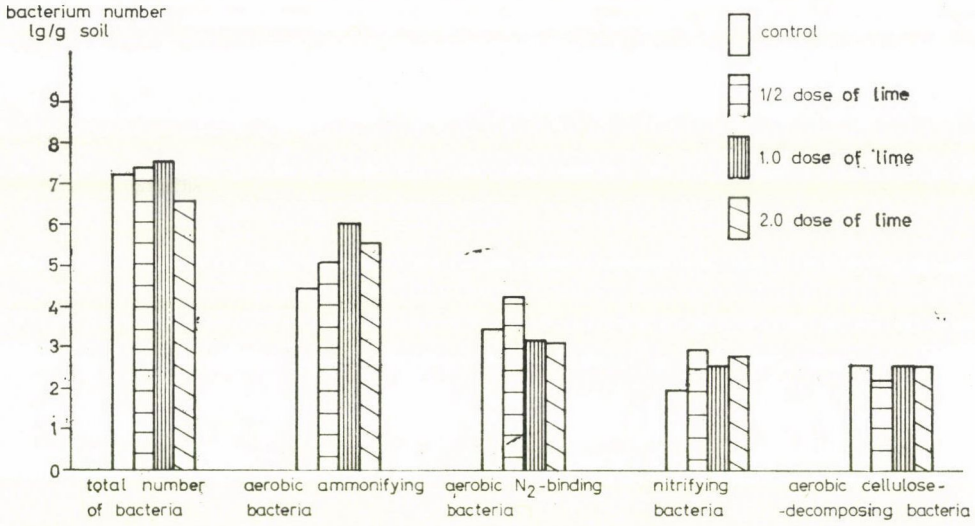


Fig. 6. **Number of bacteria belonging to different physiological groups in the soil (pot experiment, 22. 3. 1976); clay-washed brown forest soil, Kenyeri**

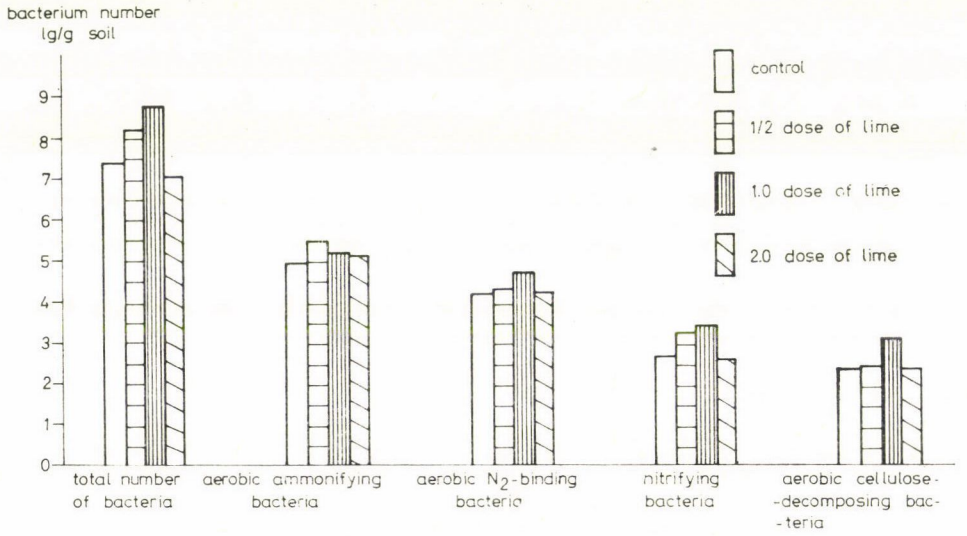


Fig. 7. **Number of bacteria belonging to different physiological groups in the soil (pot experiment, 22. 3. 1976); clay-washed brown forest soil, Nagykanizsa**

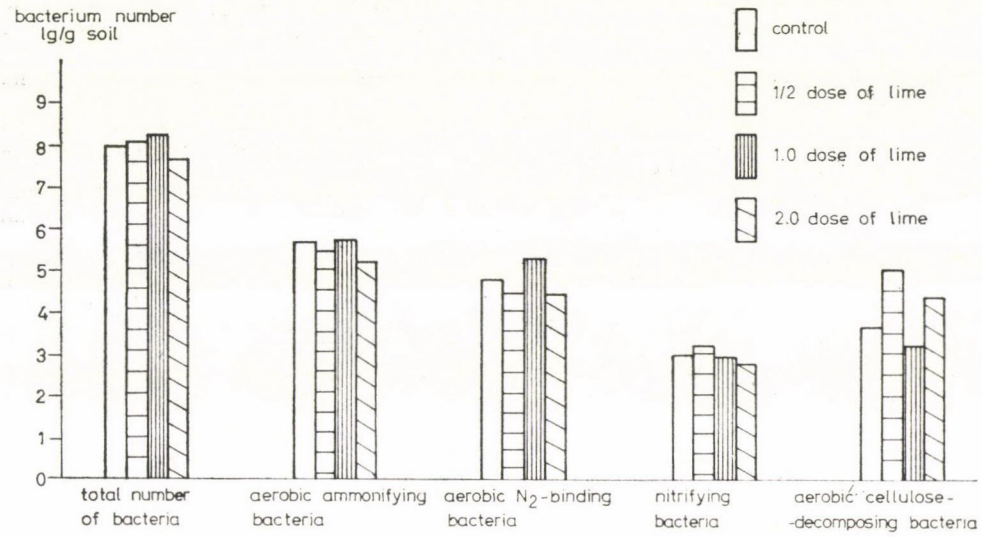


Fig. 8. **Number of bacteria belonging to various physiological groups in the soil (pot experiment, 22. 3. 1976); meadow soil, Hosszúhát**

were the highest when the 1.0 dose of lime was added to the soil, while the nitrifying and aerobic cellulose-decomposing bacteria reached a maximum when using the 1/2 dose of lime.

To sum up what has gone before, as regards the first part of the experiment it can be established that in clay-washed brown forest soils liming always results in an increase in the number of bacteria compared to the control. In the case of meadow soils the total number of bacteria and the number of aerobic N_2-binding bacteria were the highest in the control, while bacteria belonging to the other three physiological groups increased in number in response to liming.

As regards the second part of the experiment it can be stated unequivocally that maximum bacterium numbers were obtained with the 1/2 and 1.0 doses of lime, which were higher in each case than with the 2.0 dose. On the basis of these results it thus seems probable that the use of a double dose (2.0) of lime is unnecessary, as the bacteria display maximum activities even under the influence of smaller amounts of lime.

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SCALE INSECTS IN THE PARKS AND AVENUES OF BUDAPEST

By

S. Bognár, G. Vinis

UNIVERSITY OF HORTICULTURE, DEPARTMENT OF PLANT PROTECTION; METROPOLITAN HORTICULTURAL COMPANY, BUDAPEST

During an assessment of scale contamination in the parks and avenues of Budapest 45 scale species were found to be occasional or regular pests on 79 different ornamental trees and shrubs at the sites examined. Five of the 45 species (*Heliococcus bohemicus* Sulz., *Paroudablis piceae* Loew., *Pulvinaria oxyacanthae* L., *Lepidosaphes rubi* Thiem. and *Lepidosaphes crataegi* Borch.) were reported for the first time in the fauna of Hungary. Eighteen of the detected species (*Gossyparia spuria* Mod., *Pseudochermes fraxini* Kalt., *Asterolecanium minus* Russ., *A. variolosum* Ratz., *Planchonia arabidis* Sign., *Parthenolecanium corni* Bchè., *Sphaerolecanium prunastri* Fonsc., *Physokermes piceae* Schr., *Leucaspis pusilla* Loew., *Anamaspis loewi* Colv., *Lepidosaphes ulmi* L., *Lepidosaphes rubi* Thiem., *Chionaspis salicis* L., *Unaspis euonymi* Comst., *Pseudaulacaspis pentagona* Targ., *Carulaspis visci* Schr., *Epidiaspis leperii* Sign., *Quadraspidiotus perniciosus* Comst.) represent a real danger to the ornamental trees and shrubs in public places. Analysis of the components of the damaged trees and shrubs also indicated a considerable loss of water, especially during the vegetation period, as well as a certain degree of potassium deficiency caused by the scales. Hibernating forms can be sufficiently controlled by a thorough wash (with a 3—5% fruit-tree oil emulsion, or calcium sulphite in a 1 : 5 dilution) in winter. At swarming time repeated applications of organic phosphoric acid ester derivatives (0.3% "Foszfotion" containing malathion as active agent, or 0.1% Lebaycid 50 EC, a pesticide of the fention group) on at least two occasion provide satisfactory protection against scale insects on ornamental trees and shrubs in Hungary.

Introduction

With the process of urbanization and the rising cultural demands of man the plants of parks and avenues (public places) are becoming more and more important. Trees and parks established amidst the crowded streets of big cities contribute greatly to the protection of the environment. By binding the dust, purifying the air, providing shade, giving protection against noise, and last but not least by their aesthetic effect they form an integral part of our everyday life. Only healthy plants are able to fulfil these functions. The danger must be recognized in time, the organisms causing the damage identified, and the damage prevented or lessened by efficient methods of plant protection.

In the course of our investigations it was found that *Unaspis euonomy* Comst., a species of scale causing damage to the ornamental shrub *Euonymus*

japonica, was able to reduce the leaf area by as much as 0.41 m², which has implications with respect to environmental protection that can no longer be left out of consideration.

The investigations carried out so far have hardly dealt at all with the control of organisms causing damage in public places. The observations, too, have mainly been of a taxonomical character (KOSZTARAB 1959, KOZÁR 1969, 1970, 1972a, 1974, 1975). Data on the pests of ornamental trees are mostly found in the sylvicultural literature (GYÖRFI 1957, HALMÁGYI 1974), but sufficient attention has not been paid to the damage caused by scales. Literary works on the scales found in ornamental nursery-gardens and orchards (*Quadraspidiotus perniciosus* Comst., *Parthenolecanium corni* Bchè., *Sphaerolecanium prunastri* Fonsc., *Lepidosaphes ulmi* L., etc.) are more significant; they deal mostly with the ethology of the species and the possibility of control (BOGNÁR—HUZIÁN 1974, JABLONOWSKI 1916, KOZÁR 1970, 1971, 1972b, REICHART 1970, SZELÉNYI 1933, 1943, 1954). The damage done by scale species under the special environmental conditions of public places, and changes taking place in their habits have not been discussed so far in separate works written on this particular subject by Hungarian authors.

The present investigation was aimed at identifying the scale species occurring in the parks and avenues of Budapest *(Homoptera—Coccoidea),* establishing the density at which they occur, assessing the damage, and finding a possible means of control under the given conditions.

Material and Method

Species occuring in the parks and avenues of Budapest were identified by carrying out regular observations, collecting material and making microscope sections, as well as on the basis of herbarium samples and the works of BORCHSENIUS (1949, 1950, 1957, 1960) and BALACHOWSKY—MESNIL (1935). Five categories were set up according to the density of occurrence and the extent of damage:

I = the species occurs sporadically with a low number of individuals;
II = the species occurs generally with a low number of individuals;
III = the species occurs in some places (5—8) with a large number of individuals;
IV = the species occurs generally with a medium number of individuals;
V = the species occurs generally with a large number of individuals.

A control experiment was set up on Margit Island with a winter treatment of thorough washing, and an application of pesticides containing organic phosphoric acid ester during the vegetation period. The plant protection technology was worked out using the results of a three year chemical treatment of severely affected plants.

To determine the extent of qualitative losses comparative studies were carried out on the N, P_2O_5, K_2O and water contents of contaminated and healthy plants.

Results

Having identified the scale species some of them were found to be unknown in Budapest (+) or in the fauna of Hungary (++). The scale species

identified feed on 79 host plants. The extent of damage and the occurrence are shown by the Roman numerals given in brackets (I—V).

Family *Pseudococcidae*

Phenacoccus aceris Geoffr.: ***Fraxinus excelsior*** (II), ***F. ornus*** (I), ***Aesculus hippocastanum*** (II), ***A. carnea*** (I), ***A. octandra*** (I), ***Acer platanoides*** (II), ***A. campestre*** (II), ***Celtis occidentalis*** (I), ***Tilia argentea*** (II), *T.* sp. (I), ***Crataegus monogyna*** (I), ***C. oxyacantha*** (II), ***Ulmus*** sp. (I), ***Ostrya carpinifolia***$^{+}$ (I), ***Betula pendula*** (I), ***Cydonia oblonga*** (I);
Polystomophora ostiaplurima Kirb.: ***Aesculus hippocastanum*** (I);
Heliococcus bohemicus Sulz.$^{++}$: ***Aesculus hippocastanum*** ♀ 13.4.76.;
Paroudablis piceae Loew.$^{++}$: ***Picea abies*** ♀ 11.4.76., ***Picea pungens*** ♀ 29.7.76.

Fig. 1. Gossyparia spuria Mod. on an *Ulmus* sp. branch (Photo: G. Vinis)

Family *Eriococcidae*

Acanthococcus aceris Sign.: *Acer platanoidea* "Schwedleri" (I), *A. platanoides* (II), *A. campestre* (II), *Aesculus hippocastanum*$^{+}$ (II), *Acer pseudoplatanus* (I), (Fig. 2);
Gossyparia spuria Mod.: *Ulmus scabra* (IV), *U.* sp. (IV); (Fig. 1.)
Pseudochermes fraxini Kalt.: *Fraxinus excelsior* (IV), *F. ornus* (III).

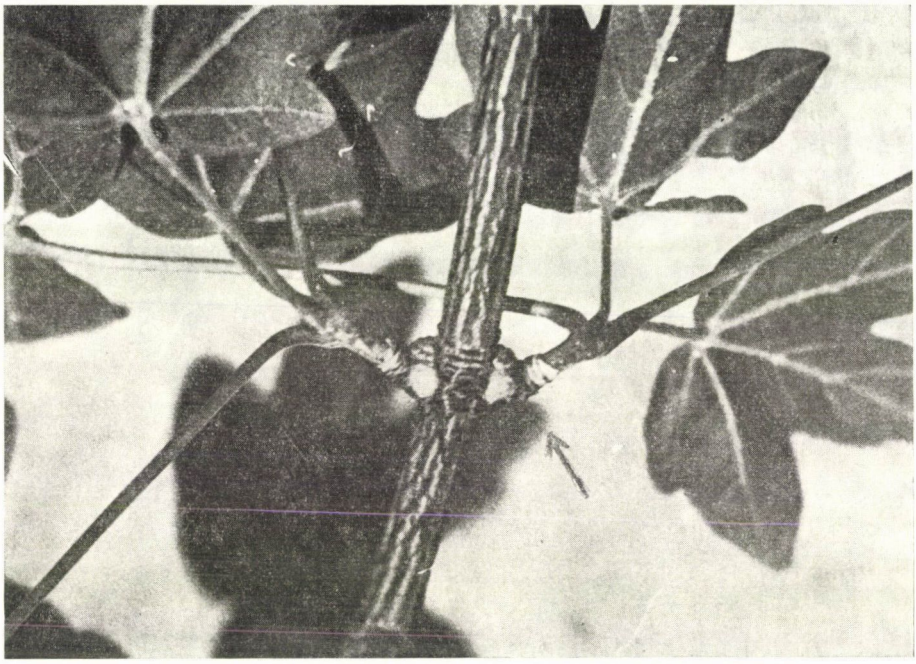

Fig. 2. Acanthococcus aceris Sign. on an *Acer campestris* shoot (Photo: G. Vinis)

Family *Kermococcidae*

Kermococcus quercus L.: *Quercus robur* (II), *Qu.* sp. (II).

Family *Asterolecaniidae*

Asterolecanium minus Russ.: *Quercus robur* (IV);
Asterolecanium variolosum Ratz.: *Quercus robur* (III);
Planchonia arabidis Sign.: *Hedera helix* (III).

Family *Coccidae*

Parthenolecanium corni Bchè.: *Acer negundo* (III), *Aesculus hippocastanum* (I), *A. carnea* (I), *A. octandra* (I), *Berberis julianae* (II), *Cornus mas* (I), *Corylus*

avellana (II), *Cotoneaster horizontalis* (II), *Carpinis betulus* (I), *Celtis occidentalis* (II), *Crataegus monogyna* (II), *C. oxyacantha* (II), *Cydonia* sp. (I), *Fraxinus excelsior* (II), *F. ornus* (I), *Hedera helix* (I), *Juglans regia* (I), *J. nigra* (I), *Lonicera tatarica* (I), *Platanus acerifolia* (I), *Populus nigra* (II), *P. nigra* "Italica" (II), *Pyracantha coccinea* (III), *Quercus robur* (II), *Robinia pseudoacacia* (III), *R. globosa* (III), *Prunus cerasifera* (II), *P. cerasifera* "Atropurpurea" (II), *Padus avium* (II), *Pyrus* sp. (I), *Eleagnus angustifolia* (II), *Malus purpurea* (II), *Morus alba* (I), *Rosa* sp. (II), *Salix* sp. (II), *Sophora japonica* (I), *Symphoricarpus orbiculatus* (III), *S. albus* (III), *Syringa vulgaris* (II), *Tamarix tetranda* (III), *Tilia argentea* (III), *Ulmus* sp. (II);

Coccus hesperidum L.: *Nerium oleander* (II), *Hedera helix* (I);

Sphaerolecanium prunastri Fonsc.: *Prunus cerasifera* (V), *P. cerasifera* "Atropurpurea" (IV);

Eulecanium mali Schr.: *Corylus avellana* (III), *C. colurna* (II), *Acer platanoides* (II), *A. pseudoplatanus* (I), *Crataegus oxyacantha* (II), *Tilia argentea* (II);

Eulecanium tiliae L.: *Acer saccharinum* (I), *Tilia argentea* (II);

Eulecanium bituberculatum Targ.: *Aesculus hippocastanum* (II), *Corylus colurna* (I), (Fig. 3);

Eulecanium rufulum Ckll.: *Aesculus hippocastanum* (II), *Cornus sanguinea* (I);

Parthenolecanium fletscheri Ckll.: *Biota orientalis* (II), *Thuja occidentalis* (I);

Pulvinaria vitis L.: *Vitis silvestris* (I);

Pulvinaria betulae L.: *Aesculus hippocastanum* (I), *Cydonia oblonga* (I), *Platanus acerifolia* (I), *Tilia argentea* (I);

Pulvinaria oxyacanthae L.$^{++}$: *Crataegus oxyacantha* "Pauli" (I);

Saissetia oleae Bern.: *Nerium oleander* (III);

Physokermes hemicryphus Dalm.: *Picea abies* (III);

Physokermes piceae Schr.: *Piceae pungens* (III), *P. pungens* "Kosteriana" (II);

Physokermes inopinatus Danz.-Koz.: *Picea pungens* (II), *P. pungens* "Kosteriana" (I).

Family *Diaspididae*

Leucaspis pusilla Loew.: *Pinus silvestris* (III), *P. mugo* (II), *P. nigra* (II);

Leucaspis pini Hart.: *Pinus nigra* (II), *P. mugo* (I);

Anamaspis loewi Colv.: *Pinus nigra* (III), *P. silvestris* (III), *P. mugo* (II);

Lepidosaphes ulmi L.: *Acer saccharinum* (II), *A. negundo* (II), *Aesculus hippocastanum* (II), *Celtis occidentalis* (II), *Crataegus oxyacantha* (II), *C. monogyna* (I), *Fraxinus excelsior* (III), *F. ornus* (III), *Malus purpurea* (II), *Pyrus* sp. (II), *Populus nigra* (III), *P. nigra* "Italica" (III), *Morus alba* (I), *Quercus robur* (II), *Q. tuneri* var. *pseudoturneri* (I), *Prunus cerasifera* (II), *Populus simonii* (II), *Berberis vulgaris* (II), *Spirea salicifolia* (II), *Syringa vulgaris* (I);

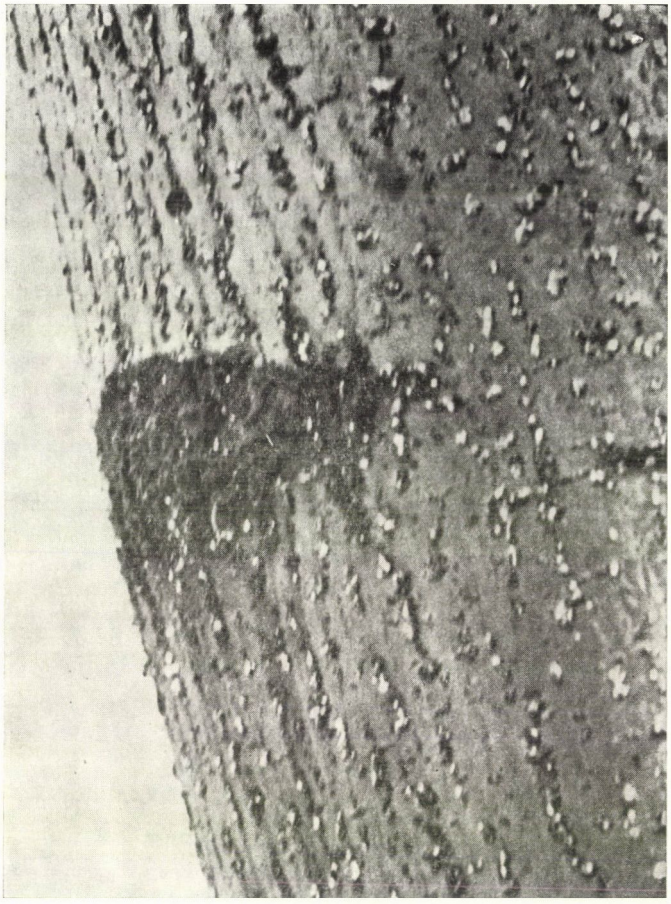

Fig. 3. Eulecanium bituberculatum Targ. on *Aesculus hippocastanum* (Photo: G. Vinis)

Lepidosaphes conchiformis Gmel.: *Morus alba* (I), *Ulmus scabra* (II), *Fraxinus excelsior* (II), *F. ornus* (I);
Lepidosaphes newstaedi Sulz.: *Pinus nigra* (II);
Lepidosaphes rubi Thiem^{++}: *Tilia argentea* (III);
Lepidosaphes crataegi Borch.$^{++}$: *Crataegus oxyacantha* (III);
Chionaspis salicis L.: *Fraxinus excelsior* (IV), *Populus nigra* (II), *P. nigra* "Italica" (I), *P. simonii* (IV), *P. alba* (I), *Salix* sp. (II);
Unaspis euonymi Comst.: *Euonymus (= Evonymus) japonica* (V), *E. europaea* (IV), *E. nana* (III), *E. fortunei* "Carlierei" (III), *Celastrus scandens* (I);
Aulacaspis rosae Bouc.: *Rosa* sp. (II);
Pseudaulacaspis pentagona Targ.$^{+}$: *Sophora japonica* (II);
Carulaspis visci Schrk.: *Juniperus communis* (V), *J. sabina* (IV), *Thuja occidentalis* (II);

Epidiaspis leperii Sign.: *Pyrus communis* (IV), *P. betulifolia* (I), *P. sativa* **(III)**, ***Sorbus aria*** (III), *S. aucuparia* (III);
Quadraspidiotus gigas Thiem et Gerne.: *Populus nigra* (II), *Fraxinus* ***excelsior*** (II);
Quadraspidiotus zonatus Frauenf.: *Quercus robur* (III), *Q. cerris* (I);

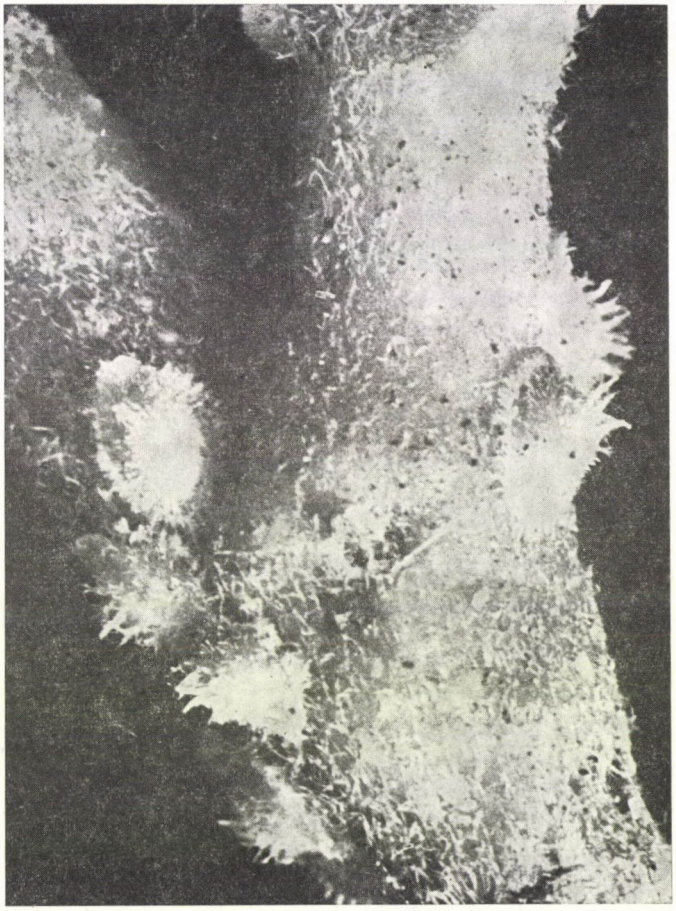

Fig. 4. Pseudochermes fraxini Kalt. on a *Fraxinus excelsior* trunk (Photo: G. Vinis)

Quadraspidiotus ostreaeformis Curt.: *Fraxinus excelsior* (II), *F. ornus* (I), ***Malus purpurea*** (III), *Ostrya carpinifolia* (III), *Populus nigra* (III), *P. simonii* **(II)**, *Syringa vulgaris* (II), *Ulmus* sp. (I);
Quadraspidiotus perniciosus Comst.: *Aesculus hippocastanum* (I), ***Berberis vulgaris*** **(II)**, *B. thunbergii* "Atropurpurea" (I), *B. julianae* (III), ***Cotoneaster horizontalis*** (III), *Crataegus monogyna* (III), *C. oxyacantha* "Pauli" (V), ***Euonymus europaea*** (I), *Malus floribunda* (V), *M. spectabilis* (IV), *M.* sp. (IV), ***Prunus***

cerasifera (IV), *P. cerasifera* "Atropurpurea" (III), *P.* sp. (III), ***Pyracantha coccinea*** (V), *Pyrus* sp. (I), *Rosa* sp. (II), *Sorbus aria* (IV), *S. aucuparia* (V), ***Salix*** sp. (II), *Ulmus* sp. (II);
Quadraspidiotus marani Zahr.: *Fraxinus excelsior* (II).

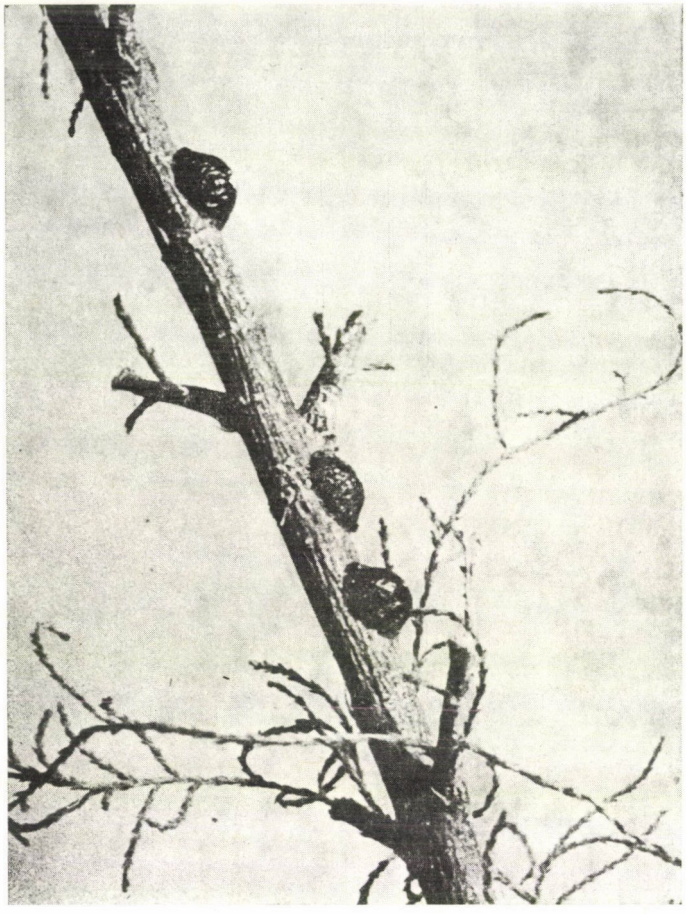

Fig. 5. Planchonia arabidis Sign. on a section of *Hedera helix* shoot (Photo: G. Vinis)

Apart from these 45 species, a scale species belonging to the *Pseudococcidae,* which lives on the trunk of the *Ulmus* sp., has not been identified so far. There are 18 scale species which occur most frequently in the parks of Budapest and cause damage from time to time.

Gossyparia spuria Mod., which produces one generation a year (Fig. 1), overwinters as a larva on the elm-tree. *Pseudochermes fraxini* Kalt. occurs in large numbers on the trunks of ash-trees (Fig. 4). This species produces one generation a year and overwinters as a larva; the larvae swarm in July. In oak

trees *Asterolecanium minus* Russ. and *A. variolosum* Ratz. cause damage. This is a one-generation species; the fully developed female overwinters, and the larvae swarm in July. *Hedera helix* is frequently damaged by *Planchonia arabidis* Sign. (Fig. 5), a one-generation scale overwintering in the egg stage. *Parthenolecanium corni* Bchè. (Fig. 6) feeds mainly on the *Tamarix, Prunus, Ulmus* and *Quercus* species and on *Robinia pseudoacacia*. It is a one-generation species overwintering as a larva; the peak swarming time for the larvae is in June. A very dangerous pest on the *Prunus cerasifera* species is *Sphaerolecanium prunastri* Fonsc.; this overwinters as a larva and the larvae swarm continuously from the end of June to the beginning of August (Fig. 7).

Fig. 6. Parthenolecanium corni Bchè. on a *Tamarix tetranda* branch (Photo: G. Vinis)

Of the evergreen plants, *Picea* is most frequently attacked by *Physokermes piceae* Schr. (Fig. 8). It overwinters as a larva; the larvae swarm at the

end of May or the beginning of June. In *Pinus* species *Leucaspis pusilla* Loew. and *Anamaspis loewi* Colv. appear in large numbers and cause serious damage. Two generations develop a year. It is mostly the sexually mature female that overwinters, though overwintering may occur in the larval form. Mass swarming of larvae takes place in May and August.

Fig. 7. Sphaerolecanium prunastri Fonsc. on a *Prunus cerasifera* branch (Photo: G. Vinis)

The damage caused by *Lepidosaphes ulmi* L. is general; in 1976 two generations developed on a number of host plants (*Crataegus monogyna, Acer saccharinum, Populus nigra, Pyrus* sp., *Salix* sp. etc.); the second swarming of larvae took place at the end of July, when male specimens also developed. Eggs were laid from the end of September to the end of October. In some places *Lepidosaphes rubi* Thiem. occurred in large numbers on lime-trees. Here it is the sexually mature female that overwinters; two generations develop a

year. The larvae first swarm from the end of May, while the second swarming can be observed up to the beginning of August. The species is heavily parasitized by ichneumons. Ash-trees, and occasionally *Populus simonii*, are severely attacked by *Chionaspis salicis* L., which according to our observations and in contrast to the literary data (BORCHSENIUS 1950) develops two generations a year. Overwintering takes place in the egg stage; the larvae swarm first in April, then at the end of July. Females developing from this swarming finish laying their eggs by the end of September. A dangerous pest on the *Euonymus*

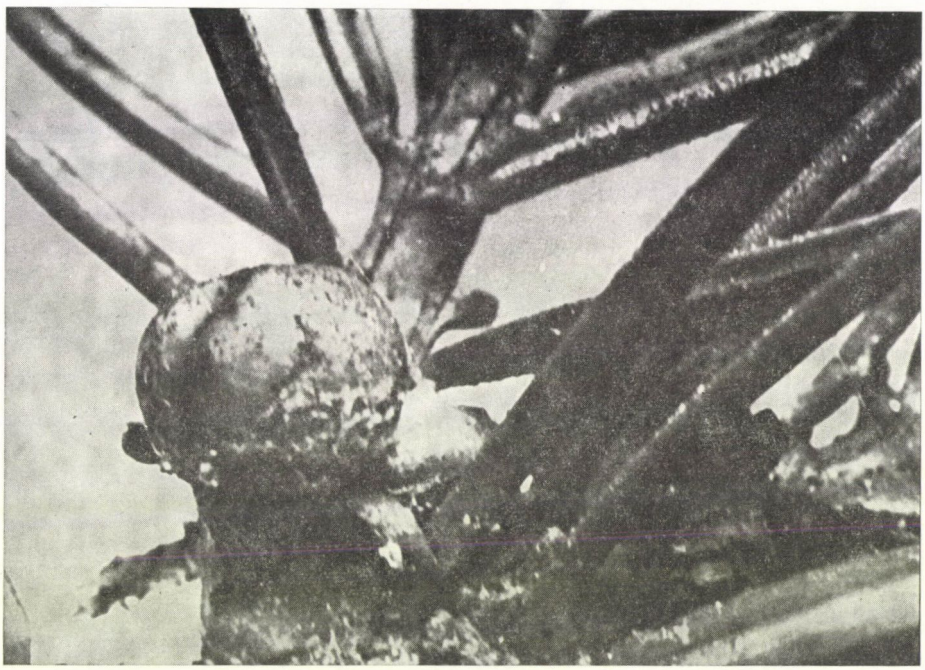

Fig. 8. Physokermes piceae Schrk. on a *Picea pungens* shoot (Photo: G. Vinis)

sp. is *Unaspis euonymi* Comst. The species mostly overwinters as sexually mature females, though the larvae may also overwinter. Two, or in part three generations develop a year. The larvae emerge gradually, so that almost all the developmental forms can be found throughout the year. Mass swarming of larvae occurs early in May, at the end of July and in September. According to our observations parasites to this species occur very seldom and in very low numbers.

Pseudaulacaspis pentagona Targ. (Fig. 9) has been found so far at three sites very distant from each other in Budapest (in districts IV, VII and XXII) on *Sophora japonica* L. The danger that the species may spread is enhanced by the fact that parasites (*Prospaltella berlesei* How.) which limit the density of

the species were not found on the contaminated plants. The species overwinters in the sexually mature, fertilized female stage. Two generations develop a year; the first swarming of larvae is in May and the second in August.

Carulaspis visci Schr. occurs in the largest numbers on the Juniperus sp. It overwinters as a sexually mature, fertilized female; the larvae swarm

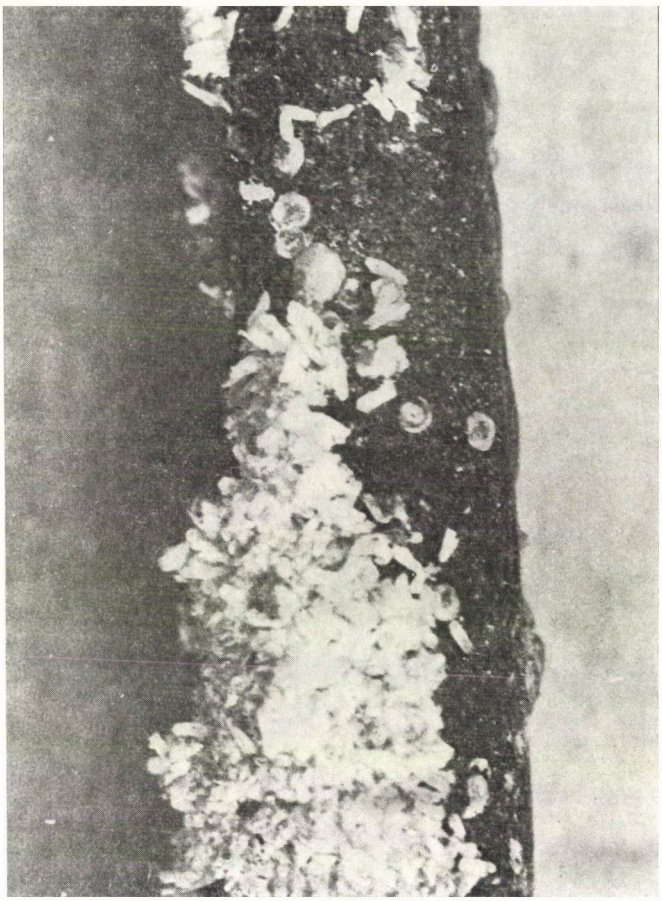

Fig. 9. Pseudaulacaspis pentagona Targ. male scales on a *Sophora japonica* branch (Photo G. Vinis)

continuously from mid-June to the beginning of August. *Epidiaspis leperii* Sign. causes damage mainly to the *Pyrus* species. The sexually mature, fertilized female overwinters, and the larvae swarm in July. The San José scale (*Quadrispidiotus perniciosus* Comst.) lives in large numbers on *Malus, Prunus, Sorbus, Crataegus* and *Pyracantha* plants. It produces two generations a year and it is usually the L_1 larva that overwinters; the first generation of larvae swarms in June and the second in August.

The dangerous nature of the above pests is increased by the fact that the different species are scattered, often being isolated from one another in the parks and avenues, which makes efficient control difficult.

In our experiments the best results were obtained by thorough washing (with a 3—5% emulsion of fruit-tree oil, or calcium sulphite diluted at a ratio of 1 : 5) used in winter against the winter forms of scale, and with pesticides containing organic phosphoric acid ester as active agent (Foszfotion 0.3%, Lebaycid 50EC 0.1%) applied on at least two occasions when the scale larvae are swarming. The effect of the chemicals can be increased by loosening the hold of the insects on the trunks and thicker branches with a wire brush before the treatment.

The analyses of plant components, aimed at establishing the extent of the damage, show (Table 1) that the scales extract a considerable amount of

Table 1

Degree of contamination by scale insects, and changes in the components of the contaminated branches

Plant (scale insect)	Degree of contamination	Date of examination	Water content, %	N	P_2O_5	K_2O
				dry matter, %		
Euonymus europaea (Unaspis euonymi)	3	Feb. 1975	54.7	0.6	0.5	0.5
E. europaea	0	Feb. 1975	54.8	1.36	0.4	0.8
E. europaea (Unaspis euonymi)	3	June 1975	38.2	1.5	1.1	0.9
E. europaea	0	June 1975	64.3	2.1	1.7	1.2
E. japonica (Unaspis euonymi)	3	Feb. 1976	50.11	1.35	0.35	0.1
E. japonica	0	Feb. 1976	52.2	1.8	1.5	0.5
Unaspis euonymi	—	Feb. 1976	69.4	6.2	1.45	2.6
Sorbus aria (Quadraspid. pern.)	3	June 1975	53.5	0.85	0.47	0.55
Sorbus aria	0	June 1975	72.0	1.07	0.50	0.61
Prunus cerasifera (Sphaerolec. prunast.)	3	June 1976	48.9	1.05	0.25	0.01
P. cerasifera	0	June 1976	57.86	1.31	0.5	0.21
Sphaerolecanium prunastri	—	May 1976	41.51	8.26	1.72	2.5
S. prunastri	—	June 1976	60.44	7.48	1.75	0.8

water especially during the vegetation period, while at the same time a certain degree of potassium deficiency occurs in the plants, i.e. the absence or a decrease in the content of potassium can be demonstrated in the contaminated plants. The plant components also vary according to the date of the examina-

tion and the development stage of the scales. Naturally, the damage is most clearly manifested in a reduction of the plant leaf area and the destruction of ornamental trees and shrubs.

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STUDIES ON THE PATHOGENEITY OF ASPERGILLUS SPECIES IN THE CASE OF CHICKEN EMBRYOGENESIS

By

O. Tóth, A. Pál, J. Nagy

UNIVERSITY OF AGRICULTURAL SCIENCES, DEPARTMENT OF PLANT PROTECTION, DEPARTMENT OF ANIMAL HUSBANDRY AND DEPARTMENT OF MATHEMATICS AND PHYSICS, DEBRECEN

In the experiment 0.1 ml of a suspension of conidia, washed off the slant agar thalli of various *Aspergillus* species using a physiological salt solution containing 1‰ Tween was inoculated into the air-cells of non-incubated soft eggs, while in the case of one species *(A. nidulans)* the vacuum infiltration of conidia was also carried out for the purposes of comparison. According to the results of the experiment the infection which occurred following the inoculation caused total necrosis in the first third of the incubation period in the case of *A. fumigatus*, *A. flavescens*, *A. fonsecaeus*, *A. parasiticus*, *A. flavus*, *A. nidulans* and *A. oryzae*, while the species *A. amstelodami*, *A. ochraceus* and *A. candidus* caused necrosis, protracted throughout the incubation period, in half the embryos. In most of the cases the clinical picture developing in the air-cell is specific to the fungus in question, while the characteristic, though very slight, changes in the different anatomical forms (e.g. accumulated matter of various colour and superficial extension below the air-cell, various extent of deliquescence, colour and consistency of the different anatomical forms, the condition of the embryo, etc.) are probably unsuitable for differential diagnoses of a praxis nature between the egg mycoses, but they are very useful for experimental purposes, and provide a means of distinguishing these infections from those caused, for example, by bacteria. The only exception is *A. nidulans*, which causes highly specific reddish pigmentation syndromes on the egg-shell membrane, very suitable for differentiation from other mycoses. When the latter species was introduced by means of vacuum infiltration the necroses were protracted, and total necrosis only occurred towards the end of the incubation period, while the pigmentation syndromes followed the larger pores and became general over the shell membrane.

Introduction

The *Aspergillus* species are cosmopolitan: they are able to live in the soil and in plant residues alike. For a long time their pathogenic activity in humans and animals was the subject of much discussion. Of the *Aspergillus* species *Aspergillus fumigatus* is now regarded most particularly as a human and zoopathogen (Fejér *et al.* 1957).

The human and zoo-pathogenic *Aspergillus* species may cause infections of the eye, skin, nail, ear, respiratory organs, digestive tract and central nervous system, and the individual species may become generalized (Bartels — Cramer 1966). According to the earlier literature the *Aspergillus* mycoses are connected with the contamination of ulcers and wounds, and their dominant character mostly appears in this way. Aspergillosis is a rare human infection

which may effect the lung, the sinusoids of the nose, the heart, as endocarditis, and even the brain. Aspergillosis may develop in the auricular canal and produce an otomycosis (ANDERSON 1957). In the course of a feeding experiment performed with goslings the fungi *Aspergillus fumigatus*, and five species which do not produce aflatoxin *(A. flavus, A. niger, A. terreus, Mucor corymbifer* and *Rhizopus nigricans)* mixed with the feed caused haemorrhages in the connective tissue under the tunica of the liver, and ulcers on the contact line of the glandular and muscular stomachs. In other cases enteritis, pericarditis, fibrinous air pockets and inflammation of the body cavity were observed without haemorrhages (PALYUSIK *et al.* 1968). In the case of pneumomycosis caused by *A. fumigatus* in ducklings yellowish-white cheesy centres compact to the touch, the size of a poppy seed or millet seed, were found sporadically in the lungs (PALYUSIK 1966). DANKÓ—SÁRI (1968) described a case of aspergillosis caused by *A. fumigatus* which was discovered in the incubator immediately after hatching. *A. fumigatus* was demonstrated in the livers, spleens, kidneys and lungs of orally infected goslings showing a 60—80% mortality (DANKÓ—TÓTH 1966). According to SZABÓ—BALÁZS (1966), when hatching duck eggs aspergillosis may reduce the hatching results to 40—50%, or even lower in the case of a serious infection.

According to KISS (1973) the rate of death caused by aspergillosis in geese exceeded 50% in the case of severe infections. SZABÓ—BALÁZS (1966) demonstrated *A. fumigatus*, and occasionally *A. flavus* and *A. citerus*, in samples taken from the air-cells and egg-shells and from various parts of the hatching room and incubators, in 90% of the cases. In our own experiments, in samples taken from various anatomical forms of goose eggs which necrotized in the first third of embryogenesis, *Aspergillus, Mucor* and *Penicillium* were obtained, together with *E. coli, Streptococcus* and *Staphylococcus* (NAGY *et al.* 1972, NAGY *et al.* 1973, PÁL 1973). When the experiments were repeated, and when the microorganisms were cultured after coagulation at high frequency as well (NAGY—PÁL 1974a, 1974b, PÁL 1975) the same microorganisms were obtained with hen eggs as are described above for goose eggs. From duck and goose eggs SZABÓ (1968) isolated mostly *A. fumigatus* in pure cultures, and sometimes demonstrated it in joint infection with *A. niger*, and with *Mucor* and *Penicillium*.

To develop an efficient protection against mycoses several factors should be known; among others we have to know when and to what extent the disease affects the embryo. It is also important to recognise the clinical picture and clarify the syndromes. In earlier investigations only the clinical picture developing in the air-cell was considered to be characteristic. The necessity of changing this view was raised by SZABÓ—BALÁZS (1966). The earlier observations dealt primarily with *A. fumigatus*, while the other *Aspergillus* species were hardly mentioned in the literature, despite the fact that the species of the *Aspergillus* genus are wide-spread, so the eggs in the incubator may be

infected by more than one species. According to our present knowledge all farm and laboratory animals are susceptible to the various fungal toxins, irrespective of whether the disease in question has been observed under natural conditions or not (PALYUSIK 1973). For this reason it was thought necessary to carry out inoculation experiments with 10 *Aspergillus* species in order to find out when and in what syndromes the damaging effect of the individual species on the embryo manifests itself.

Material and Method

In this experiment 0.1 ml of a 12,000/ml conidium suspension washed off a three week old slant agar culture of fungi (listed in the Results) using a physiological salt solution containing 1‰. Tween was inoculated into the air-cells of non-incubated hen eggs, which were placed in an incubator on the day following the inoculation. The fungi were placed at our disposal by the Ogrod Bot. Inst. Hodowli i Akl. Roslin w Bydgoszczy (Poland), with the exception of *A. oryzae*, which was obtained from the Biogál Pharmaceutical Factory, Debrecen.

The inoculation was performed in a sterile chamber, and the inoculation site was immediately covered with a paraffin layer applied with a brush. Three preliminary trials were made with the fungus species, each applied on 10 hen eggs obtained from the Debrecen Incubating Station. The control in all three trials included the same number of hen eggs inoculated with 0.1 ml physiological salt solution containing Tween in a manner similar to the conidium suspension inoculations. After the preliminary tests (the results of which were similar to those obtained in the experiment carried out with a larger number of eggs and are thus not presented here in detail) the experiment was repeated with 200 non-incubated "Hybro" eggs on each of three occasions. The number of eggs used as control was equal to that of the experimental population. In the experiment the same methods were used as in the preliminary tests.

With one of the fungus species *(A. nidulans)* the experiment was repeated by inoculating 200 non-incubated Hybro eggs in each of 8 replications with a suspension of the same conidium number as previously, by means of vacuum infiltration. The experimental apparatus consisted of a vacuum exsiccator, a vacuum gauge and a rotary air-pump. The eggs were placed in the exsiccator six at a time so that they were perfectly covered by the conidium suspension. Then the pressure was decreased, and maintained at the required level by constant pumping.

The reduction in pressure and the period for which the vacuum was maintained differed from one treatment to the other but was always of an order of magnitude which could be measured in minutes, and in most cases did not exceed the time required for the gases to leave the egg in the form of bubbles. During the period of reduced pressure the gases which were occluded in the egg departed through the egg-shell with intensive bubbling. The vacuum pump was then stopped and the pressure of the external air flowing in slowly pressed the conidium suspension into the eggs, and for 5—10 minutes they were kept under atmospheric pressure. in the suspension. Knowing the initial and final weights of each egg the amount of suspension introduced could be determined. After the introduction of the inoculum the eggs were kept at 37.5°C and examined every day by candling and a subsequent dissection according to the method of KISS (1973). Necroses found during the first two days of the hatching process were determined by the proliferation of the blascodiscus and the differentiation of the primordialis capillaris.

The reisolation of the individual fungus species and the exclusion of secondary infection by bacteria were carried out by means of control examinations after the pathological dissection.

Results

1) *Aspergillus fumigatus* (Fresen). In the first and third experiments all the two hundred eggs necrotized on the first day of hatching, while in the second experiment 140 died on the first, and 60 on the second day. The result

of dissection was uniform: the air-cell was filled with a fine, white cobweb-like thallus. From the yolk a smooth layer was deposited under the air-cell, the rest of the yolk deliquesced, and became characteristically watery and slimy. On the thin, smooth deposit of yolk around the embryo hyperaemic mucous parts were observed (Fig. 1).

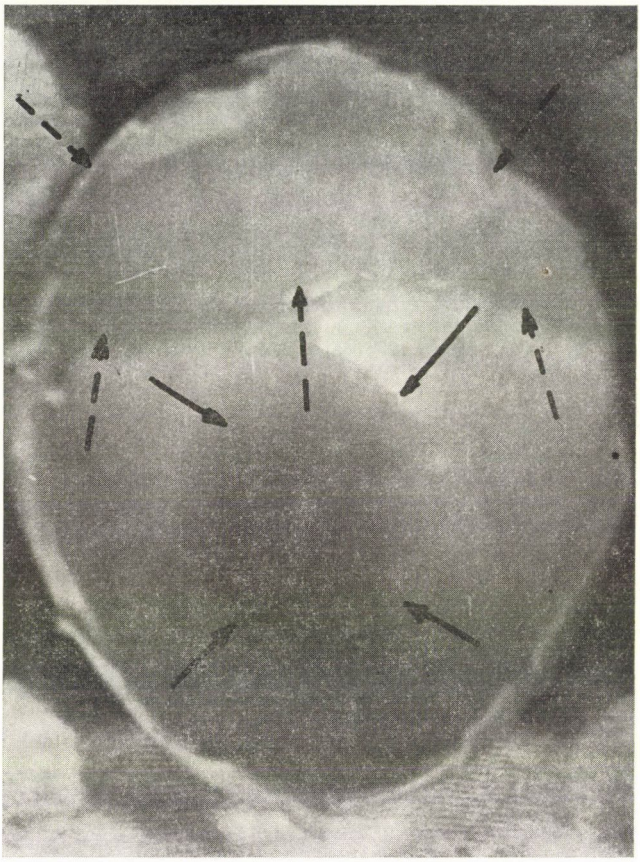

Fig. 1. Smooth surfaced deposit below the air-cell (dotted arrow) and part of a serous deliquescence (solid arrow) in a case of death caused by *A. fumigatus* on the first day of incubation

2) *Aspergillus flavescens* (Wreden). In the first and second experiment all the 200 eggs died on the first day of hatching, while in the third experiment 20 died on the first, 80 on the second, 80 on the third and 20 on the fourth day of hatching. The pathological anatomical picture is characteristic, and uniform as in the former case. The air-cell is filled with a white thallus, the whole of the yolk is deposited in a "massive curd-like layer" below the air-cell, and covered by a whitish slime. Below the deposit large brownish patches are seen on the shell membrane over the entire surface. That part of the yolk which does not

form a deposit assumes a custard-like colour and consistence and becomes deliquescent. Around the embryo hyperaemic areas are occasionally found. In the course of pathogenesis it was observed that mucosity preceded the custard-like deliquescence. In the third experiment, in eggs necrotized on the fourth day of incubation, the injection entered below the membrane surround-

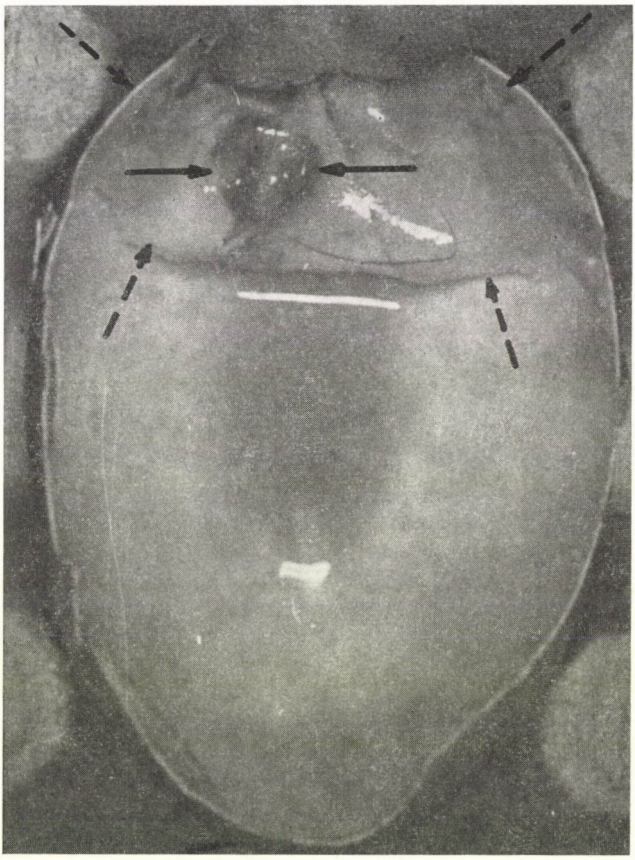

Fig. 2. In the case of death caused by *A. flavescens* the whitish-yellowish "massive curd-like" deposit below the air-cell (dotted arrow) includes the highly hyperaemic embryo (solid arrow). Death occurred on the third day of incubation

ing the air-cell, and the thallus thus developed within this membrane too, i.e. mostly outside the air-cell, and therefore the lesions described above are more strongly expressed (Fig. 2).

3) *Aspergillus fonsecaeus* (Thom et Raper). In all three experiments death occurred on the first six days of incubation in 80 –100% of cases (Table 1). The white thalli completely filled the air-cells (in most cases). The results of dissection were similar to those obtained with *A. fumigatus* (watery

Table 1

Death caused by various fungus species during the embryogenesis of the experimental groups

Species	Day			Choked	Hatched
	1—7	8—14	15—21		
Control 1	60				140
Control 2		20			180
Control 3	20				180
1. A. fumigatus					
1st experiment	200				
2nd experiment	200				
3rd experiment	200				
2. A. flavescens					
1st experiment	200				
2nd experiment	200				
3rd experiment	200				
3. A. fonsecaeus					
1st experiment	180				20
2nd experiment	200				
3rd experiment	160		20	20	
4. A. amstelodami					
1st experiment	40	60			100
2nd experiment	100			20	80
3rd experiment	100	60	20		20
5. A. ochraceus					
1st experiment	40	20			140
2nd experiment	100	60			40
3rd experiment	140	20			40
6. A. candidus					
1st experiment		20		40	140
2nd experiment	80	40			80
3rd experiment	60	40	20	40	40
7. A. parasiticus					
1st experiment	200				
2nd experiment	200				
3rd experiment	200				
8. A. flavus					
1st experiment	200				
2nd experiment	200				
3rd experiment	200				
9. A. nidulans					
1st experiment	120	80			
2nd experiment	180				20
3rd experiment	200				
10. A. oryzae					
1st experiment	200				
2nd experiment	160	20			20
3rd experiment	200				

deliquescence, mucosity, smooth deposition of yolk below the air-cell, intensive hyperaemia of the embryo). In the third experiment four specimens died on the 16th day; the capillaries interweaving the yolk were considerably dilated, the heart was round and thin-muscled, the liver showed hyperaemia and the stomach and intestines were inflamed.

4) *Aspergillus amstelodami* (Mangr.). In all three experiments 50% of the specimens died by the 13th day of incubation (Table 1). The air-cell was filled by a white thallus and the symptoms were similar to those observed with *A. fumigatus*, except that the chalaza was occasionally of a greenish opalescent shade, and a brownish deposit similar to that obtained with *A. fonsecaeus* was found on the shell membrane. In embryos older than 13 days liver hyperaemia and inflammation of the digestive tract were observed.

5) *Aspergillus ochraceus* (Wilhelm). On the first 14 days of incubation altogether two-thirds of the experimental stock of the three experiments died (Table 1). The shell membrane was covered by a mucous deposit, and there was a characteristic watery deliquescence, with hyperaemia around the embryo. Below the air-cell a small, smooth deposit was found, sometimes of a brownish colour and occasionally covered by mucus. In some cases the chalaza was of a greenish colour.

6) *Aspergillus candidus* (Link). On the first five days of incubation death did not occur in the first experiment, while 140 specimens died in the second and third experiments (Table 1). Intensive watery deliquescence and hyperaemia around the embryo were observed. In most cases there was no deposit, but occasionally a thin, smooth-surfaced deposit occurred. On the tenth to thirteenth day of incubation 100 specimens died in the three experimental groups, with watery deliquescence as the most characteristic symptom, and an occasional small brownish deposit below the air-cell, and less frequently a greenish opalescent discoloration of the chalaza; the embryo was hyperaemic in a large proportion of the cases, and the capillaries interweaving the shell membrane were considerably dilated. In the first experiment 40 specimens died of suffocation shortly before hatching, as they were unable to utilize the yolk properly; the yolk was excluded and its capillaries were highly dilated; dissection showed myocardial degeneration and hyperaemia of the liver.

7) *Aspergillus parasiticus* (Speare). The 400 specimens of the first and third experiment died on the first day of incubation, while in the second experimental group 100 died on the first day, 40 on the second and 60 on the fifth. The air-cell was filled with a greenish-yellowish-whitish thallus which caused wrinkling of the double membrane surrounding the air-cell. The yolk showed intensive mucosity, and turned into a massive, amorphous "curd-like" formation. Below the air-cell a thick, extensive, characteristically massive amorphous "curd-like" deposit was found. A definite custard-like mucosity also occurred in some cases. The embryo was mostly hyperaemic (Fig. 3).

8) *Aspergillus flavus* (Link). In all three experimental groups almost all the stock (540 specimens) died on the first day and 60 on the fifth day (Table 1). A lesion characteristic of the air-cell is the extensive white fungal thallus which fills up all the available space. The yolk was discoloured, and in part became amorphously "curd-like"; it stuck below the air-cell and was occasionally

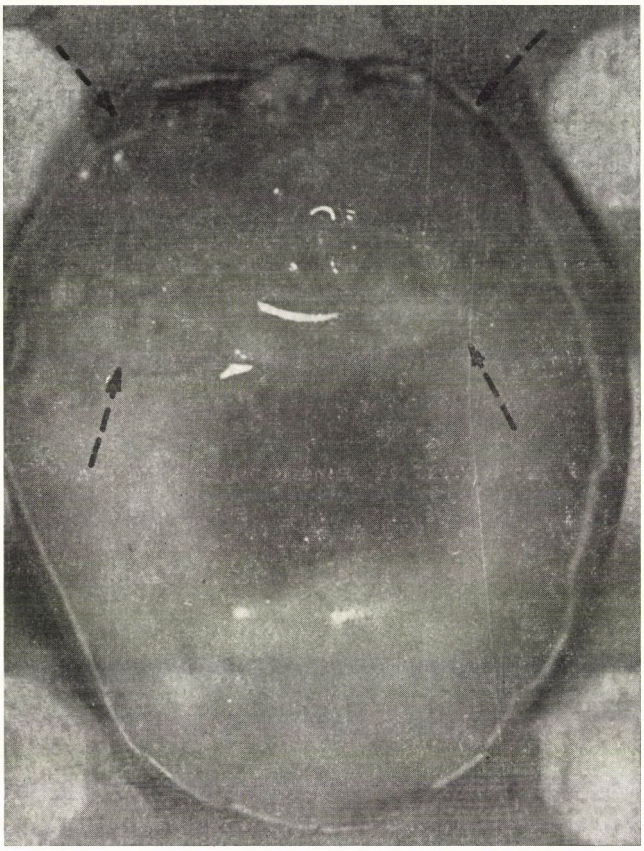

Fig. 3. Thick, extensive "amorphous, curd-like" deposit below the air-cell, caused by *A. parasiticus* (dotted arrow). Death occurred on the first day of incubation

covered by a characteristic whitish mucus. The rest of the yolk became watery, or custard-like, and deliquesced; hyperaemia around the embryo was general in some cases (Fig. 4).

9) *Aspergillus nidulans* (Eidam) Wint. Of the three groups (600 specimens) infected by injection 500 specimens died on the first seven days of incubation (Table 1). The white, thick, solid thallus was characteristic, almost "parenchymatic"; of the fungi included in the experiment this formed the most solid thallus. The yolk occasionally deliquesced in the form of a brown,

turbid fluid, while in other cases it became mucous, in addition to the other symptoms mentioned. Below the air-cell there was a narrow, smooth-surfaced deposit which surrounded the embryo; in most cases the latter was hyperaemic and covered by a whitish mucus.

In some cases the deposits extended over the entire surface of the shell membrane. After their removal a reddish pigmentation specific to *A. nidulans*

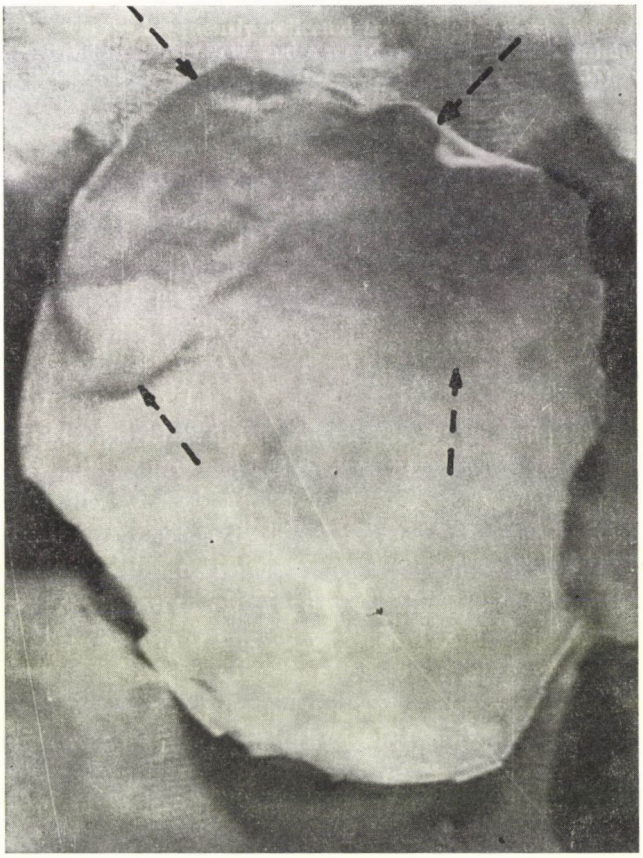

Fig. 4. "Amorphous curd-like" deposit of yolk, occasionally covered by whitish mucus, below the air-cell, caused by *A. flavus* (dotted arrow). Death occurred on the first day of incubation

was observed to extend over a large part of the shell membrane. The most characteristic feature was the uniform reddish pigmentation on the outer surface of the shell membrane forming the air-cell (at the pointed end of the egg), on the convex side of the "cap", which was visible after the removal of the smooth deposit formed by the yolk. Apart from the characteristic discoloration appearing on the yolk-side surface of the double membrane bordering the air-cell, i.e. on the "cap", the reddish pigmentation characteristic of

mycosis caused by *A. nidulans* generally occurred on the shell membrane in the form of well defined patches, and in the majority of cases formed typical reddish rings and spots (Figs 5a, 5b and 5c). Of the usual symptoms, a greenish opalescent discoloration of the chalaza also occurred.

When *A. nidulans* infection was induced by vacuum infiltration, on the average of experiments carried out in 8 replications, 15% of the deaths occurred

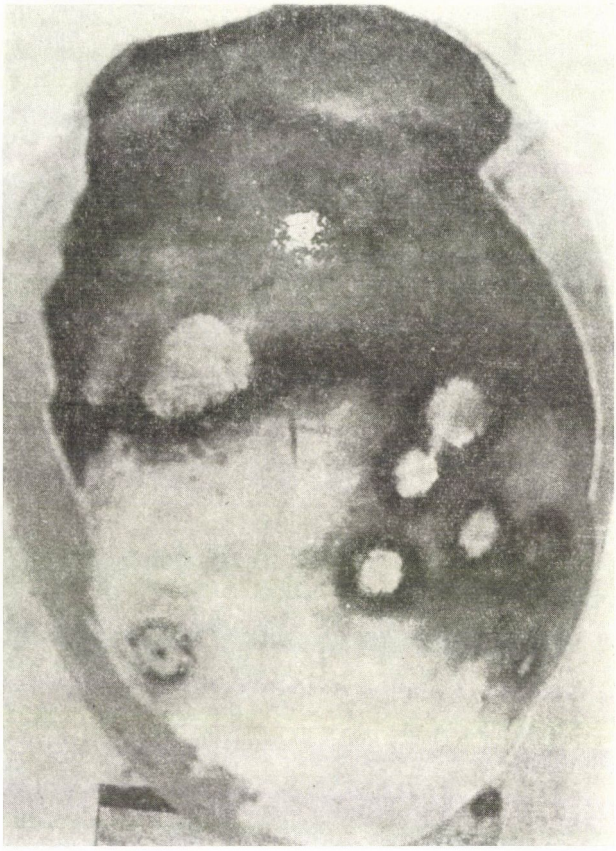

Fig. 5a. Characteristic reddish pigment stripes and rings occurring generally over the entire surface of the shell membrane, caused by *A. nidulans* introduced by injection. Death occurred on the third day of incubation

between the first and seventh day of incubation, 80% on the fourteenth to eighteenth day, and only 5% reached the postnatal stage. Reddish pigmentation on the shell membrane as described above could be observed in postnatally surviving specimens as well, and in certain cases a reddish pigmentation of the outer cover was mostly observed (pars abdominalis, Fig. 6). Specimens reaching the postnatal stage (5% of the population in the case of vacuum infiltra-

tion) were unviable almost without exception. In those specimens which broke through the egg-shell and hatched umbical openness and occasionally partial ectopy were found, while those which only reached the stage of fissuring did not resorb the yolk and consequently showed complete ectopy, or in more

Fig. 5b. Characteristic syndromes of reddish pigment produced by *A. nidulans* introduced by injection: spots, stripes and rings on the surface of the shell membrane

serious cases — owing to the closing of the abdominal wall — the yolk was detached and finally excluded. The reddish pigmentation spread over the entire surface of the shell membrane in a dotted pattern corresponding to the pores (Figs 6b, 6c).

When examining the types of lesions in specimens infected by injection and vacuum infiltration we found that in the first case the pigmentation was localized between the blunt end of the egg and the equatorial plane, while

when the eggs were infected by vacuum infiltration the pigmentation extended over the total surface of the shell membrane (Figs 6a, 6b, 6c).

10) *Aspergillus oryzae* (Ahlburg). During the first 3 days of incubation 560 of the 600 specimens included in the experiment died (Table 1). In the air-cell a compact white thallus developed, which wrinkled the membrane towards the sharp end of the egg and filled out the available space. Around the embryo typical hyperaemia was found, the yolk became clondy and watery and

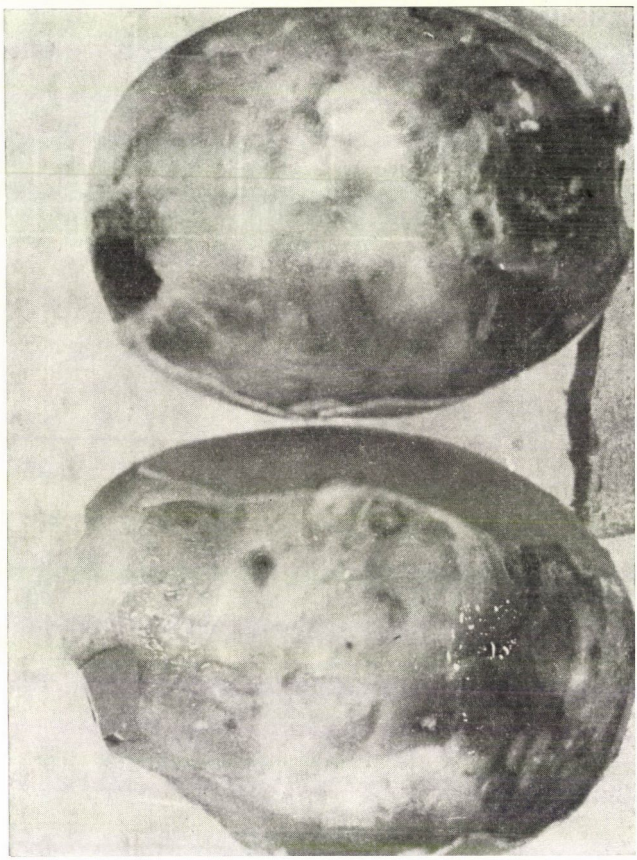

Fig. 5c. Characteristic reddish pigment spots on the surface of the shell membrane between the equatorial plane and the blunt end of the egg, caused by *A. nidulans* inoculum introduced by injection. Death occurred on the first day

deliquesced, an opalescent or whitish mucosity occasionally occurred, and the embryo stuck to the shell membrane. Less frequently an insignificant, hardly perceptible smooth deposit was observed below the air-cell.

Discussion

According to PALYUSIK (1966) the suction effect developing while the egg is cooling down enables the spores and hyphae of the fungus to enter the air-cell through the pores of the egg-shell in a mechanical way. Almost the same opinion on the mechanism of infection is held by SZABÓ—BALÁZS (1966), who consider that the hypha penetrates into the inside of the egg through the

Fig. 6a. Reddish pigmentation on the outer cover caused caudo-ventrally and pectorally by *A. nidulans* introduced by means of vacuum infiltration before incubation. Death occurred on the 19th day of incubation

pores of the egg-shell. The thin white of egg between the double membrane may play a role in this, depending mainly on the temperature and the air humidity. The fact that true solutions and suspensions are equally able to penetrate the egg-shell was proved by KISS *et al.* (1973) in isotope experiments, bioactive tests and with the staining reaction, thus confirming the

mechanism of mycosis infection described above. With such an infection mechanism the embryo sometimes continued to develop and even to hatch despite the presence of the thallus of *A. fumigatus* in the air-cell. SZABÓ (1968) infected hen eggs with *A. fumigatus* spores mixed with sterilized saw-dust, placed them in a thermostat at a temperature of 37°C for one day and obtained only 10—25% germination. The natural infection of the egg is most accurately

Fig. 6b. Dotted pigmentation caused by *A. nidulans* suspension introduced by means of vacuum infiltration and forming a reddish stripe of pigment below the equatorial plane. Death occurred on the 16th day of incubation

imitated by rubbing it with conidia mixed in some litter material, e.g. saw-dust. The method has the disadvantage of requiring a high number of experimental specimens (owing to the low — 10—25% — extent of infection).

This gives information primarily on the efficiency of inoculation, rather than on the stage of embryogenesis at which the conidia or hyphae of different species reaching the air-cell cause death. This is why the method of injecting

into the air-cell a conidium suspension of a quantity determined in preliminary experiments was chosen. After injection into the air-cell was completed, the opening at the blunt end of the egg was closed with paraffin according to the method of MÉSZÁROS (1960), which proved to be suitable for the embryos used as control. The procedure requires great care and can be carried out only in a sterile chamber; even under such conditions there is the possibility of con-

Fig. 6c. Dotted pigmentation caused by *A. nidulans* suspension introduced by means of vacuum infiltration as in the previous figure; it has become general over the entire surface of the shell membrane. Death occurred on the 16th day of incubation

tamination by other fungi. In the control group of the first experiment 40 embryos died on the seventh day, presumably due to natural infection contracted in the nesting-box; in the second experiment 20 embryos died on the eight day. Inoculation was the most successful in the control group of the third experiment, where 20 specimens were lost owing to sterility.

PERLAKI *et al.* (1959) are of the opinion that the green, red and black rots are the consequences of bacterial decomposition. In most cases lesions caused by fungi are difficult to distinguish from those caused by bacteria, as they often occur together. According to KISS (1962) fungi which attack the egg-shell may promote the penetration of bacteria into the inside of the egg by growing through the pores of the egg-shell with their hyphae, thus making way for the bacteria. When infecting duck eggs with *A. fumigatus* SZABÓ—BALÁZS (1966) found that the clinical picture develops in the air-cell in 99% of the cases, as the thalli need a great deal of oxygen in order to develop. In a case of high mortality when hatching in June they found a fairly large, greyish-green, loose, soft coating in the air-cell. According to SZABÓ (1968) in the case of infection by *A. fumigatus* candling usually shows a lentil-size spot over the air-cell; sometimes the whole air-cell is filled with mould, while in other cases the latter occurs only along the tiny cracks of the egg-shell in the form of spots or lines. In the course of a control by candling WEINER (1968) found a lentil- to nut-size spot in the air-cell on the 16th to 18th day of incubation, which later developed into a greyish-green thallus covering the whole surface of the air-cell and choking the embryo. In our own experiments deposits of yolk formed on the shell membrane due to the effect of high frequency and direct current caused death by suffocation just as the fungal thalli did (NAGY—PÁL 1972). WEINER (1968) sometimes observed an easily removed layer of mould on the hatched chicks. He further found (WEINER 1968) that bacterial infection may become associated with lesions caused by fungi but the phenomenon may also occur in the opposite order.

Our experiments were aimed at imitating the phase of natural infection when the penetration of the fungus precedes the incubation and then begins to grow under the influence of temperature and humidity. According to our observations death in the first third of the incubation period may be caused by *A. fumigatus, A. flavescens, A. fonsecaeus, A. parasiticus, A. flavus, A. nidulans* or *A. oryzae*. In each of such eggs thalli showing morphological features specific to the fungus and completely filling the air-cell develop, similarly to the results obtained by the above mentioned authors with *A. fumigatus*, while the pathomorphological lesions developing from the different anatomical forms fall within the sphere of finer distinctions, and in most cases are probably unsuitable for the differential diagnosis of deaths caused by fungi; the single exception is *A. nidulans*, which causes a highly characteristic pigmentation of the "cap", as well as pigment spots and rings. It is inferred that when the inoculum is introduced by injection the stripes and spots of pigment develop around the larger capillary networks, while the rings follow the course of the capillaries which surround the large-size pores of the egg-shell. The reddish pigmentation appearing on the shell membrane following experimental infection by means of vacuum infiltration marks the larger pores with spots as above, with the dif-

ference that — unlike the above cases — it becomes general over the entire surface of the shell membrane. There are no data in the relevant literature on the pathogeneity of *A. nidulans* observed in practical hatching, although KARASSZON—TÓTH (1959) refer to its zoopathogeneity. Necrose caused by mycotoxicosis are characteristic in the different anatomical forms as well as in the clinical picture developing in the air-cell, and are easily distinguished from death caused by bacteria, avitaminosis, genetic factors, etc. by their characteristic symptoms, e.g. various deposits below the air-cell, serosity, mucosity, discoloration of the shell membrane, etc. Necroses caused by *A. amstelodami*, *A. ochraceus* and *A. candidus* took place at various times during the incubation period and in most cases the result of hatching was 50%. Thalli completely filling the air-cell only occurred with *A. amstelodami*, while in the case of *A. ochraceus* and *A. candidus* the conidium generally did not start to develop, probably due to the fact that the incubation temperature did not coincide with the optimum heat requirement of the fungus (FEJÉR *et al.* 1957). The lesions caused in the yolk and in the thin and thick layer of egg-white are nearly identical with those caused by the other fungus species included in the experiment. Under farm conditions these three *Aspergillus* species probably have little importance in reducing the hatching ratio.

In those experimental groups where death occurred during the last phase of embryogenesis, dissection revealed hyperaemic liver, inflammation of the whole digestive tract, occasional myocardial degeneration, unused and sometimes excluded yolk, on which the capillaries were highly dilated. In a later aero-ionization — ozone therapy experiment (unpublished, but reported in a lecture NAGY—PÁL 1973, PÁL 1977) similar lesions were observed in specimens which had changed over to pulmonary respiration and died of ozone toxicosis during the last few days of incubation. Without carrying out toxicological examinations it would be difficult to decide whether death was due to suffocation caused by the thallus filling the air-cell, or to toxicosis caused by the toxin produced by the fungus. In a feeding experiment carried out with different fungus species on day-old goslings PALYUSIK *et al.* (1968) observed myocardial degeneration, ulcers in the muscular stomach and on the border of the muscular stomach and the small intestines, and in other cases haemorrhages under the membrane of the liver; these symptoms are much more serious than those observed at the embryo stage.

Conclusion

From the results of our experiments it can be established that when *A. fumigatus*, *A. flavescens*, *A. fonsecaeus*, *A. parasiticus*, *A. flavus*, *A. nidulans* and *A. oryzae* penetrate the egg-shell and the shell membrane they cause the

death of the chicken embryo in the first third of the incubation period, while *A. amstelodami, A. ochraceus* and *A. candidus* induce necrosis in half the experimental stock, protracted throughout the whole period of incubation. In most cases the clinical picture developing in the air-cell is specific for the fungus and the fungus can be reisolated, while the characteristic but tiny lesions of the anatomical forms are probably unsuitable for the practical differential diagnosis of egg mycoses, though excellent for experimental purposes and in separating mycoses from other infections, e.g. of bacterial origin. *A. nidulans* is the only exception, producing a specific reddish pigment appearing in a patch on the "cap" and as spots, stripes and rings on the shell membrane and is highly suitable for distinguishing this mycosis from others.

According to WEINER (1968) in some hatching stations mould appeared in spite of all protective measures. SZABÓ—BALÁZS (1966) found the damage caused by *Aspergillus* species to appear more and more frequently, often despite increased caution, and they observed that the diseases could not always be prevented by strictly hygienic methods. They are of the opinion that in both farms and hatching stations, in addition to the strictest hygienic measures a centrally managed active treatment of the breeding eggs before incubation is needed, which can best be solved at the hatching stations. According to KISS (1964) any method that destroys the reproductive organs of the fungi only on the outer surface of the egg-shell (e.g. UV irradiation, washing with fungicides, etc.) provides only a partial control of mycoses.

It seems that satisfactory results can only be expected from fungicide or fungistatic preparations introduced through the egg-shell. Experimental results relevant to this question will be published later.

Acknowledgements

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DISTRIBUTION AND TRANSPORT OF INDOLEACETIC ACID IN GREEN AND ETIOLATED BEAN SHOOTS

By

M. VARGA, ZS. SZENDRŐ

JÓZSEF ATTILA UNIVERSITY, DEPARTMENT OF PLANT PHYSIOLOGY, SZEGED

The IAA contents in the shoots of bean seedlings grown in light and dark do not substantially differ in spite of the great difference in stem elongation. Thus, illumination does not influence the total amount of free IAA, though there are great differences between green and etiolated plants as regards the distribution of IAA within the shoot. In etiolated shoots the larger part of the total IAA content is transported to the hypocotyls, and only a minor part is found at the site of synthesis, in the apical part; in green shoots, on the other hand, the situation is reversed. The intensity of the basipetal transport of ^{14}C-IAA applied to primary leaves substantially decreases in light and increases in dark, in green and etiolated intact shoots alike. Light promotes the conjugation of IAA molecules into immobile IAA-aspartate and IAA-glucosides in the course of transport. On the basis of the results it can be established that it is the extent of basipetal IAA transport rather than the IAA content that is in proportion to the stem elongation in green and etiolated bean shoots.

Introduction

The question of what causes the intensive stem elongation which is a characteristic phenomenon of etiolation has not yet been properly settled. According to earlier views phytohormones, primarily auxin, which cause cell elongation are found in larger quantities in etiolated plants or plant parts than in green plants (plant parts) developing in light. On the other hand, many authors found no differences in the IAA contents of shoots and shoot parts exposed to light and dark, or observed a light-induced increase in the IAA content. The confusion is further increased by contradictory data concerning the light dependence of IAA synthesis and metabolism. The view which states that the increased longitudinal growth of the etiolated plants is due to the higher auxin content of the shoot is thus disputable.

On the basis of the above the idea arose that the difference in stem elongation between green and etiolated shoots is due to the effect of light and dark on the basipetal auxin transport rather than to their effect on the IAA content. Unfortunately, however, the demonstration of the light inhibition of basipetal IAA transport has been confined almost exclusively to the transport of IAA taken up from an agar block donor in segments excised from stems or coleoptiles, and very little attention has been paid so far to questions concerning the light dependence of endogenous auxin transport in intact plants.

As an initial approach to the solution of this problem our investigations were aimed at determining the total IAA content in intact shoots of bean seedlings grown in light and dark and its distribution in the different parts of the shoot, at examining the intensity of auxin transport in intact shoots in light and dark, and at comparing these data with the extent of stem elongation.

Material and Method

The "Kompolti fehér gyöngy" variety of *Phaseolus vulgaris* L. was used in the experiments. The seeds were sown in perlite moistened to 70% of the water capacity, and supplied with a measured amount of Prjanisnikov culture solution. Some of the seedlings were grown in a light chamber at a temperature of 24°C and an illumination of 8000 lux; the rest were kept in a dark chamber at the same temperature.

The growth of the green and etiolated shoots was followed for 8 days after emergence by daily measurements of the stem length and of the fresh and dry weights of shoots and shoot parts.

IAA was extracted from the tissue homogenizate with cold methanol: the fat content and the pigments were removed from the extract by shaking it three times with petroleum ether. The fraction extractable with methanol in 6—24 hours contains the free IAA and the IAA-conjugates which together make up 95—96% of the total IAA content of the tissues (VARGA—BITÓ 1968). The indole compounds were separated by thin-layer chromatography on a silica gel G (Merck) layer, using chloroform-ethylacetate-formic acid (5 : 4 : 1) and isopropanol-7% ammonia-water (8 : 1 : 1) as solvent, as described in the above-mentioned work. The indole compounds were identified on the basis of R_f values compared to those of the synthetic compounds, the colour obtained with the Ehrlich and Salkowski reagent, the UV fluorescence and UV absorption spectra.

The quantitative determination of free IAA and IAA-conjugates was carried out by UV spectrophotometry. The respective chromatogram spots were scraped off the plate and eluated with methanol; the quantity of material contained in the eluate was determined with a Specord UV VIS apparatus at 280 nm. For each sample 12—15 chromatograms were processed.

The protein content was determined by the turbidimetric method, using standard serum albumin, after BAGI—FARKAS (1967).

Carboxyl-^{14}C-IAA was used in studying the basipetal auxin transport in light and dark. The autoradiograms were prepared on Forte High Speed X-ray film with a 7-day exposition.

The examinations were performed in four replications each.

Results

Stem elongations of green and etiolated seedlings. The hypocotyls of seedlings grown in light and dark were still of the same average length on emerging from the soil (4th day); on the 5th to 8th day, however, the lengths of the green hypocotyls decreased to 72, 51, 49 and 43% of those of the etiolated analogues (Fig. 1). Differences in growth were also manifest in the fresh weight (Table 1). The average weights of the intact shoots and excised hypocotyls of the etiolated seedlings were larger than those of the green seedlings in spite of the lower dry matter weights of the former (Table 2). The higher fresh weights of the etiolated shoots thus seems to be due to a higher water content, which is probably the result of the increased water uptake associated with more intensive cell elongation.

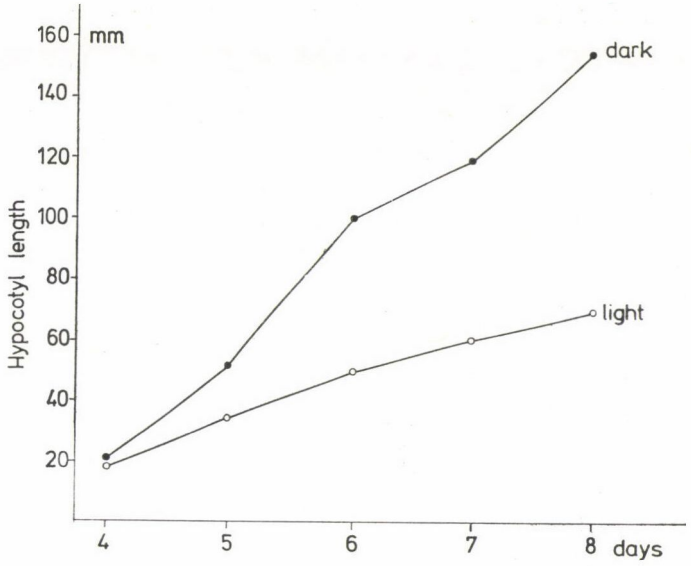

Fig. 1. **Longitudinal growth of bean shoots in light and dark (n = 4.10)**

Table 1

Average fresh weights of green and etiolated bean shoot parts
(L = light, D = dark)

Day	Whole shoot		Hypocotyl		Apical part	
	L	D	L	D	L	D
5	0.268	0.400	0.214	0.370	0.060	0.030
6	0.406	0.786	0.299	0.682	0.096	0.061
7	0.550	1.060	0.300	0.960	0.220	0.050
8	0.625	1.300	0.330	1.120	0.282	0.154

Table 2

Percentage dry weight in various parts of green and etiolated bean shoots
(L = light, D = dark)

Day	Whole shoot		Hypocotyl		Apical part	
	L	D	L	D	L	D
5	5.36	5.87	5.31	4.76	16.82	17.18
6	8.98	6.25	5.55	4.31	18.09	18.47
7	9.04	6.87	7.49	5.33	19.01	18.41
8	10.04	7.23	8.92	6.41	20.74	20.50

Protein content in green and etiolated shoots. It was necessary to determine the protein content so that the IAA concentration of shoots and shoot parts could be expressed per mg protein as well. Although at the age of 7 days the seedlings still live mostly on the nutrient reserves of the cotyledons, the protein content is nevertheless higher in shoots grown in light than in etiolated shoots (Fig. 2). According to the data the higher protein content in the green shoots is mainly due to the higher protein levels of the apical meristem and the young leaves, because the hypocotyls of green and etiolated shoots show hardly any difference in protein content.

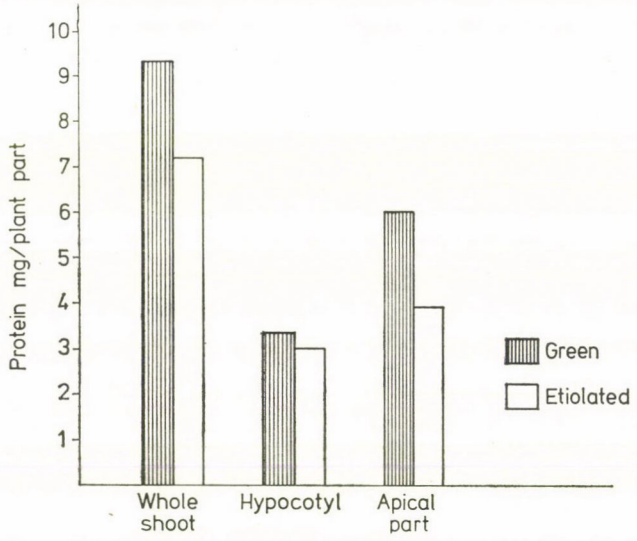

Fig. 2. Protein content in 7-day-old bean shoots

The amount of free IAA and its distribution in green and etiolated shoots. In different parts of bean shoots developed in light and dark, the IAA concentration (the amount of IAA per unit weight) and the IAA content (the amount of IAA per plant part) were both determined; in this way conclusions can be drawn from different points of view. According to the data the IAA concentration per unit fresh weight (Fig. 3) is higher in the shorter green shoots than in the etiolated ones; this difference is almost certainly due to the larger fresh weight of the latter. As for the difference in IAA concentration between the basal (hypocotyl) and apical (terminal bud + primary leaves) parts of the shoot, it has been found that in plants grown in light the amount of IAA is smaller in the hypocotyl, and significantly larger in the apical part, while in the case of dark grown plants the situation is reversed.

Different conditions are found for the whole shoot when the amount of free IAA is related to mg protein (Table 3); in this case the IAA concentration

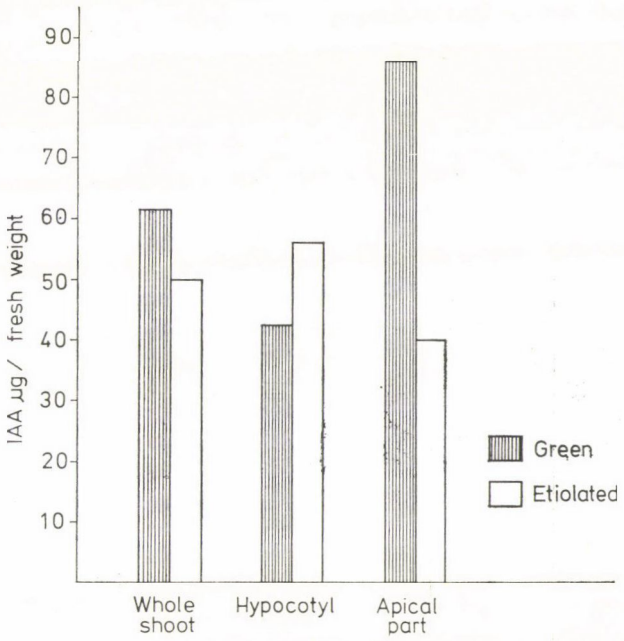

Fig. 3. **IAA concentration in 7-day-old bean shoots and their parts (related to fresh weight)**

Table 3

IAA concentration in 7-day-old bean shoots and their parts
(calculated as protein)

	µg IAA/mg protein		
	Whole shoot	**Hypocotyl**	**Apical part**
Green	2.28	3.34	1.70
Etiolated	2.93	5.60	1.06

proves to be higher in the etiolated shoot. The obvious reason for this is the lower protein content of the etiolated shoots compared to the green ones. Nevertheless, as regards the distribution of IAA between the basal and apical parts of the shoot the same holds true as in the case of fresh weight.

When analysing the different plant parts for free IAA content no significant difference was found between the total IAA contents of whole green and etiolated shoots, but the distribution of auxin between the apical and basal parts was significantly different. Fig. 4 shows that in light grown plants more than half (54.3%) of the free IAA content of the shoot was found in the hypocotyl, with less (45.7%) in the apical part; in dark grown plants, on the other hand, 79.2% of the IAA content was transported to the highly elongated

hypocotyls, and only 20.8% remained at the site of the synthesis, in the apical part. Thus the highly elongated hypocotyls contain substantially more free auxin than the shorter green ones.

The amount and distribution of IAA-conjugates in shoots of bean seedlings grown in light and dark. Of the IAA-conjugates a small amount of indoleacetyl aspartate and large amounts of IAA-glucosides (indoleacetyl-β-D-glucose

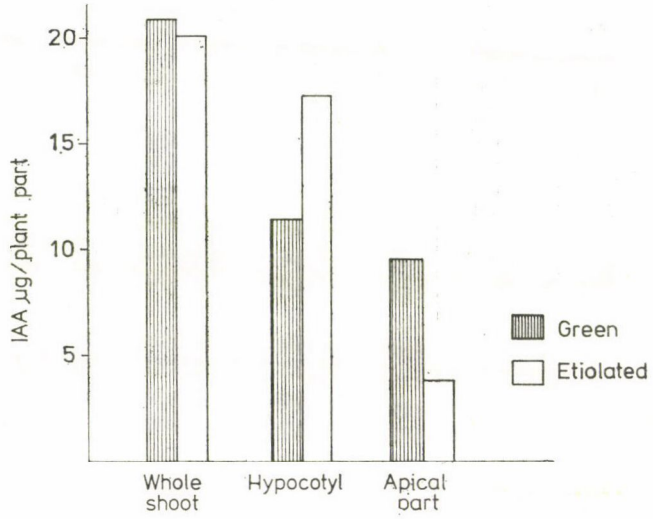

Fig. 4. IAA content in 7-day-old bean shoots and their parts (related to plant parts)

and arabinose) were found in the bean shoots. Both kinds of conjugate were found in larger quantities in green than in etiolated intact shoots. From the data shown in Fig. 5 it can be established that in light grown plants a larger proportion of the total amount of IAA-aspartate and IAA glucosides was localized in the hypocotyls (58.1 and 61.3%, respectively), and a smaller proportion in the apices (41.9 and 38.7%, respectively); while in the dark grown plants the reverse was true (40.8 and 45.2, and 59.2 and 54.8%, respectively). It thus seems that light promotes the conjugation of free IAA molecules into immobile forms both at the site of the synthesis, in the apical meristems, and in the course of transport.

Basipetal transport of ^{14}C-IAA in light and dark. The specific activity of the carboxyl-^{14}C-IAA used in the experiments was 3.65 mCi/mM. From the 4.10^{-3} M stock solution an amount containing 0.60 µCi activity was dropped on the primary leaves of 7-day-old green and etiolated shoots, after which some of the plants were exposed to light and the others were kept in the dark. After 1—6 hours shoots taken as samples were destroyed in hot methanol and dried, after which autoradiograms were prepared from them.

The differences in the rate of auxin transport taking place in non-etiolated shoots in light and dark are shown in Figs 6 and 7. In light little or no radioactivity was transported in 2 hours from the green primary leaves to the stem. After 3 hours the activity appeared in the upper part of the epicotyl, and 6 hours were required for the hypocotyl to become fully labelled (Fig. 6). In dark, on the other hand, the basipetal transport of ^{14}C-IAA proceeded at a much

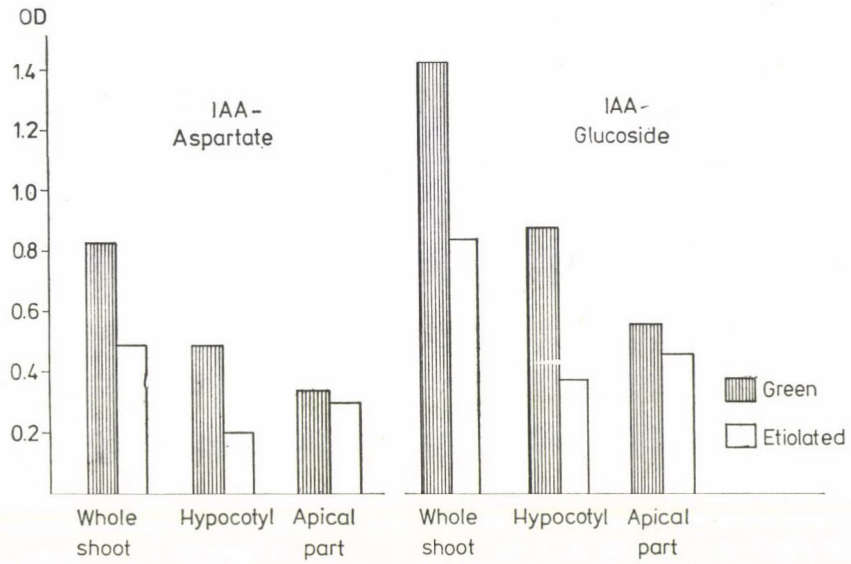

Fig. 5. Amount of IAA conjugates in 7-day-old bean shoots and their parts (related to plant parts)

higher rate and to a greater extent than in light: in shoots of the same length radioactivity appeared in 2—3 hours in the lowermost part of the hypocotyls (Fig. 7). Light thus has a decisive inhibitory effect on the basipetal movement of IAA in green shoots.

The intensity of auxin transport in etiolated shoots was influenced in the same way by illumination (Fig. 8). In the 3rd hour of the ^{14}C-IAA basipetal movement the activity was only distributed in the leaves and epicotyl, and hardly appeared in the hypocotyl. In the dark, on the other hand, almost the whole of the labelled IAA was transported in this time from the leaves and accumulated in the hypocotyl. Thus, the basipetal transport of auxin is considerably inhibited by light in etiolated shoots as well.

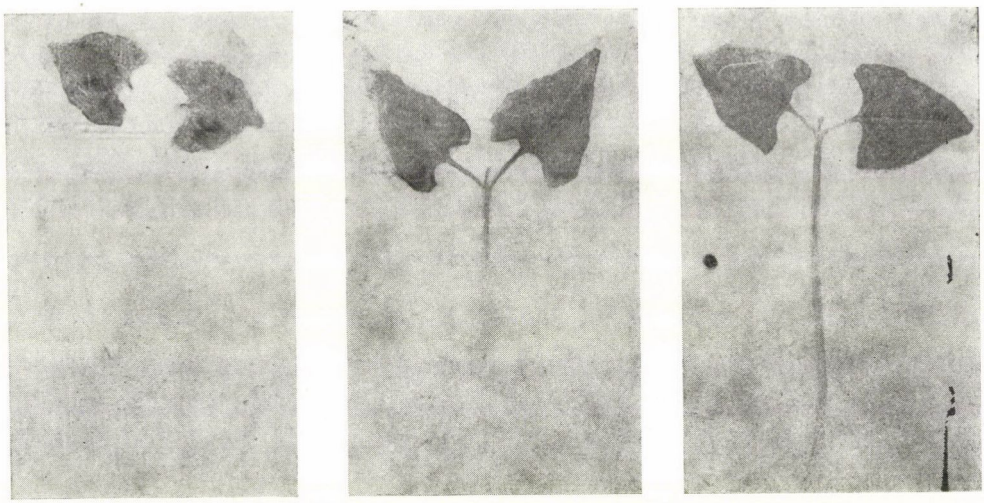

Fig. 6. Basipetal transport of ^{14}C-IAA applied on the primary leaves of the green shoot, in light

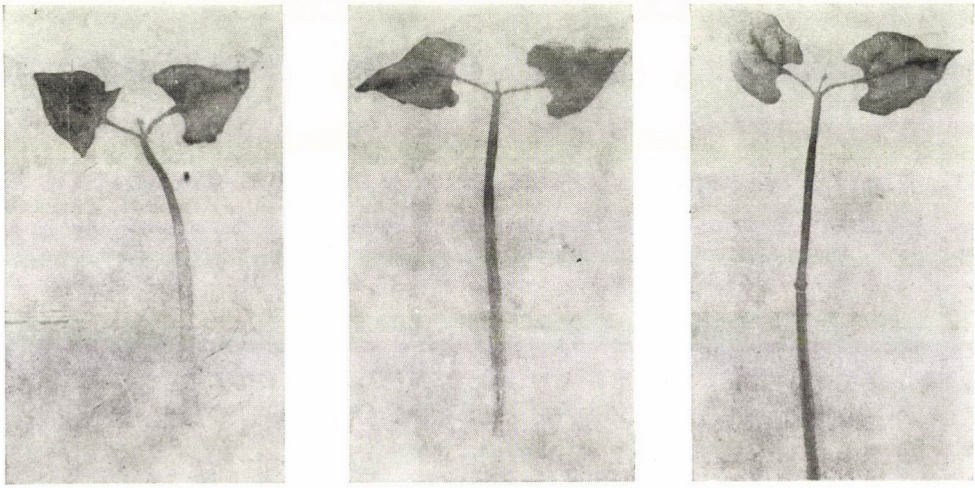

Fig. 7. Basipetal transport of ^{14}C-IAA applied on the primary leaves of the green shoot, in dark

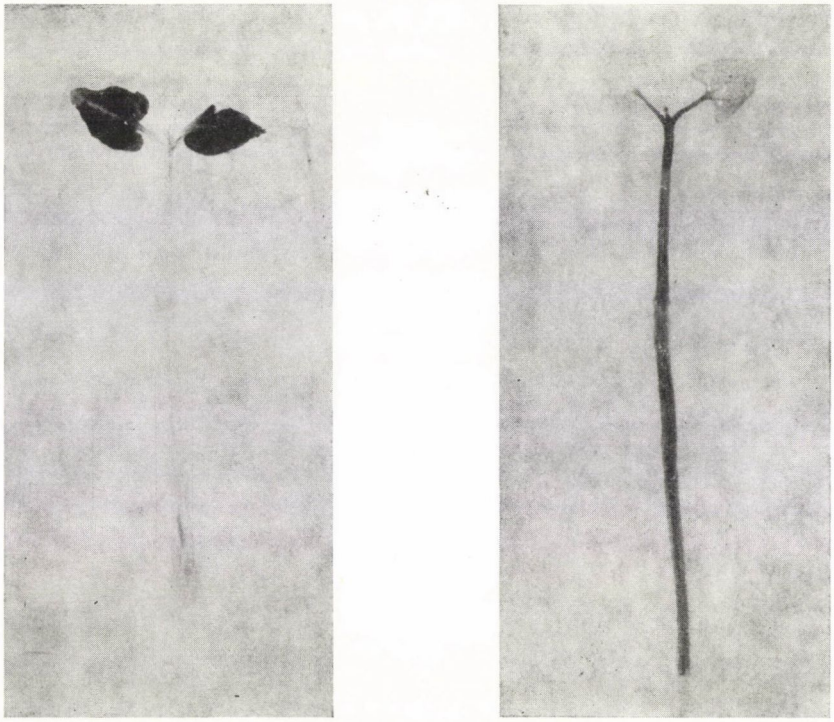

Fig. 8. Intensity of the basipetal transport of ^{14}C-IAA applied on the primary leaves of the etiolated shoot, in light (L) and in dark (D)
(Autoradiograms prepared after 3 hours of auxin movement)

Discussion

Some authors found a linear correlation between the shoot length and the IAA content of plants grown in light and dark (VON GUTTENBERG—ZETSCHE 1956, FLETCHER—ZALIK 1964); however, according to our experimental data the increased elongation of etiolated bean shoots cannot be attributed to the accumulation of a larger quantity of IAA in the dark. On the contrary, in whole shoots the concentration of IAA related to fresh weight is higher in light, and what is still more important: with the measured amount of auxin calculated for plant organs there is no substantial difference between the free IAA content of green and etiolated shoots. Our results agree with the data of SHEN MILLER—GORDON (1966a, b) who did not find a significant dif-

ference between the total auxin content in the irradiated and shaded sides of oat and maize coleoptiles.

The main sites of IAA synthesis are in the apical meristem and in the young leaves. Most analyses concerning the light dependence of IAA biosynthesis demonstrated that the process was stimulated by light (LIVERMAN 1955, SHEN MILLER—GORDON 1966b, AGHION *et al.* 1966, MOORE—SHANER 1967), which again makes it doubtful whether the intensive stem elongation of the etiolated plants can be explained by the higher auxin level.

A large part (about half) of the IAA produced in the apical part of the shoot is transported basipetally in the form of active and mobile free acid (VARGA 1968). The light inhibition of the intensity of the basipetally polar IAA transport has been demonstrated by many authors in pea epicotyl, oat coleoptile and segments of these (THIMANN—WARDLAW 1963, SHEN MILLER—GORDON 1966a, b, NAQVI—GORDON 1967, THORNTON—THIMANN 1967, HAGER—SCHMIDT 1968a, SHEN MILLER *et al.* 1969, NAQVI 1975); the opposite has been reported by only a few authors (VON GUTTENBERG—ZETSCHE 1956, KOEVENIG—JACOBS 1972). The experiments with intact bean shoots described in the present paper also confirm that the basipetal transport of ^{14}C-IAA is considerably inhibited by light.

Very little is known as yet about the mechanism of the auxin transport-decreasing effect of light; detailed studies are required on the subject. The fact that in the light the activity of IAA-oxidase is more intensive than in the dark (VARGA 1968, RUSSEL—GALSTON 1969), which means that more IAA can be inactivated in the course of transport, may have something to do with this effect. In connection with 3-methylene-oxindole, one of the main products of the oxidative destruction of IAA, HAGER—SCHMIDT (1968a, b) established that it inhibited cell elongation and blocked the auxin flow, supposedly by hindering the active secretion of IAA molecules from the cell. According to our results more IAA is conjugated into immobile IAA-aspartate and IAA-glucoside forms in light grown bean seedlings than in those raised in the dark, which may also contribute to the decrease in auxin movement in light. A similar result was obtained by MORRIS (1970), who found that the production of IAA-aspartate was stimulated by light, as well as by LANTICAN—MUIR (1969) who reported the stimulating effect of light on the activity of indoleacetyl aspartate synthetase.

Our experimental data show that although bean shoots developing in light and dark do not differ in the total amount of free IAA, they are quite different as regards its distribution in the basal and apical parts. In etiolated shoots some 80% of the free IAA content is transported from the apex to the hypocotyl, while in green shoots only half as much (47%) is transported as in dark grown plants, and the larger part remains in the apical part of the shoot. To sum up, it can be established that in green and etiolated shoots it is the

extent of IAA transport rather than the IAA content which is in proportion to stem elongation.

In connection with photomorphosis, investigations on the light-dependence of IAA synthesis and metabolism are now in progress.

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VARIA

EFFECT OF IRRIGATION ON FLOWERING IN THE SOUR CHERRY VARIETY "ÉRDI BŐTERMŐ"

The influence of cultural practices, including irrigation, on flowering has not been studied so far.

When choosing pollinating varieties it is important to know the time of flowering. Investigations into the microphenophases of flowering phenomena — e.g. the viability of the pistil and the daily course of dehiscence in the anthers — provide the possibility of choosing simultaneously flowering varieties in a more reliable manner.

At the Érd-Elvira station of the Horticultural Research Institute we examined the effect of irrigation on flowering in the sour cherry variety "Érdi bőtermő" in 1974—1975.

The orchard was planted in 1970 at a spacing of 6×4 m, with *Prunus maheleb* as rootstock; the trees were trained to normal spindle form. The sour cherry variety "Érdi bőtermő" was produced by Pál Maliga from "Pándy meggy" crossed with the Large English variety. It is a selfing variety ripening 8—10 days before "Pándy meggy". Each of the treatments used in the experiment was replicated four times; each plot was planted with six grafts.

The flowering phenomena were studied every day on individually numbered flowers, using a previously elaborated flowering dynamics observation method (NYÉKI—IFJÚ 1975).

Flowering was studied with a dynamical observation method in three replications by marking 100 flowers per treatment. The treatments were: inter-row cultivated, non-irrigated; inter-row cultivated, irrigated; inter-row grassed, non-irrigated and inter-row grassed, irrigated.

Irrigation was carried out based on a weekly exsiccator determination of the water content of the soil. In the irrigated treatments the water content of the soil was kept above 50% of the available water. At each irrigation the 0—60 cm layer of the soil was saturated to full water capacity. Accordingly, irrigation was carried out four times in 1973, and five times in 1974. In April 1974 the soil moisture was higher than 50% of the available water, due mainly to the satisfactory conditions of winter precipitation. Substantial differences in the water content of the soil occurred during the summer of each year, so flowering was primarily influenced by the water supply in the previous year. The water supply was the poorest in the non-irrigated treatment sown with grass between the rows, where water was required not only by the trees but also by the grass.

The effect of irrigation on the condition of the trees. The influence of the various treatments on the condition of the trees can be seen in Table 1.

The table shows that in both years the number of flowers on the examined grafts was lowest in the inter-row grassed, non-irrigated treatment, and highest in the inter-row cultivated irrigated treatment. Irrigation increased the number of inflorescences and flowers in both treatments compared to the non-irrigated treatments.

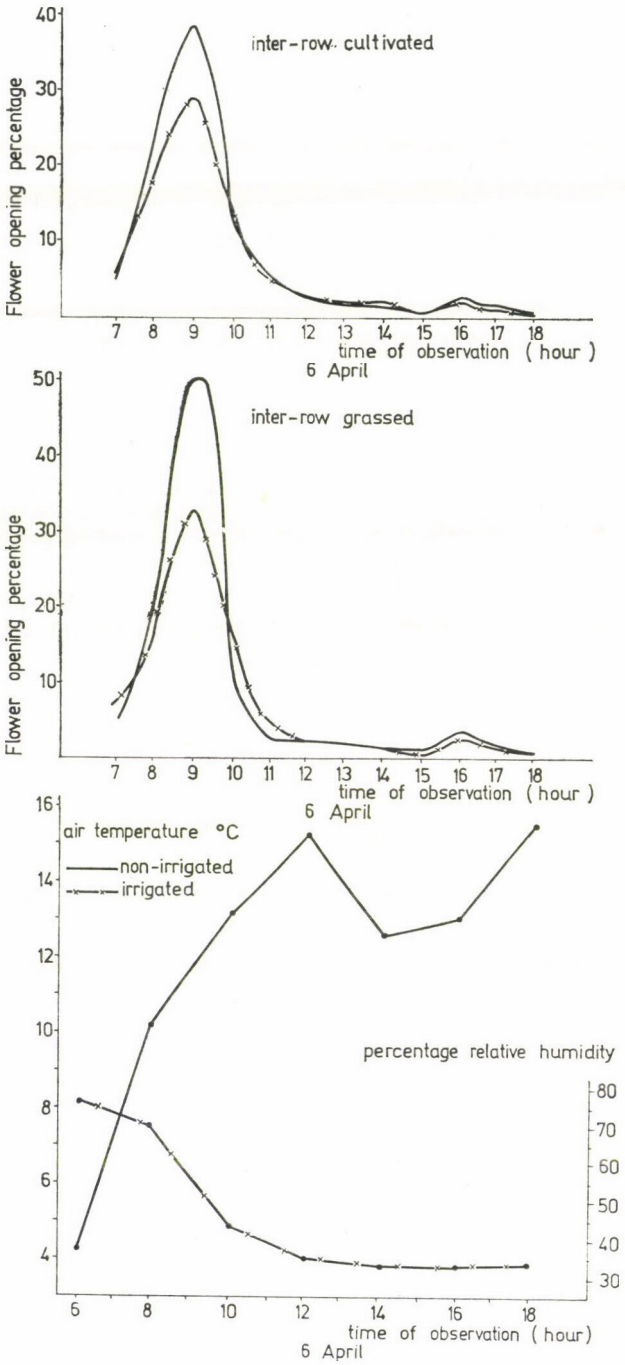

Fig. 1. Effects of irrigation and temperature on the daily course of flower opening in the sour cherry variety "Érdi bőtermő" (1974, Érd-Elvira)

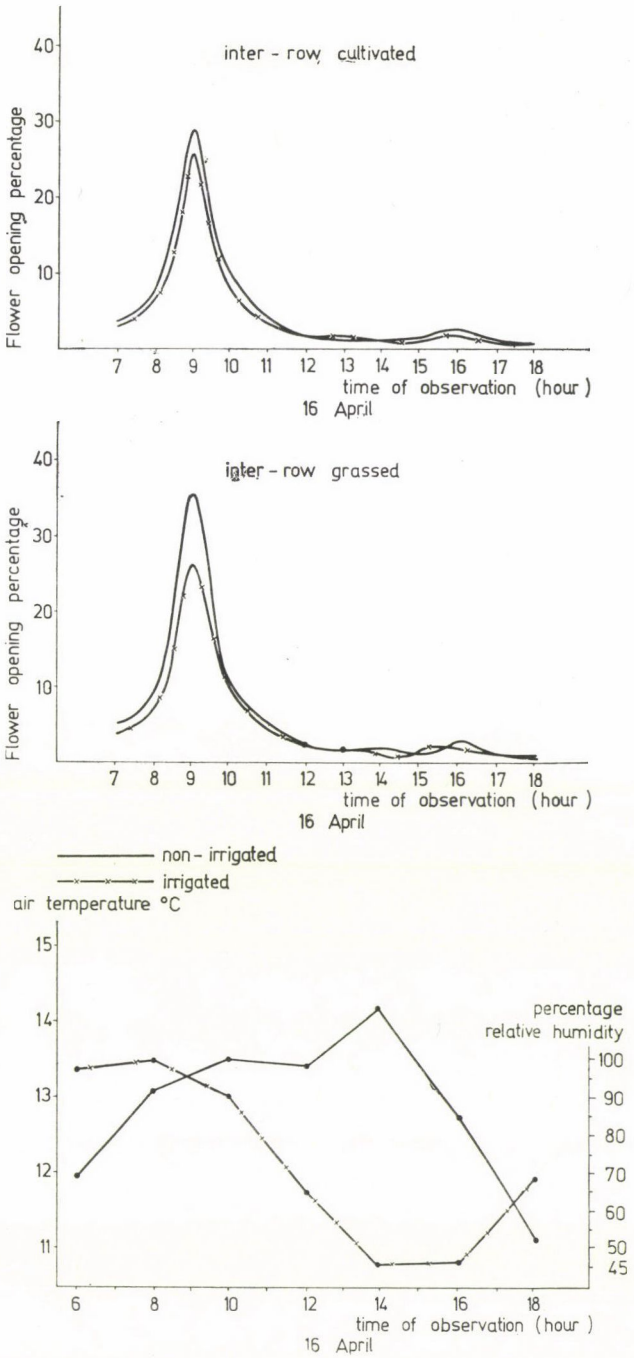

Fig. 2. **Effects of irrigation and temperature on the daily course of flower opening in the sour cherry variety "Érdi bőtermő" (1975, Érd-Elvira)**

Table 1

Effect of irrigation on the extent of flowering and girth in the sour cherry variety "Érdi bőtermő" (1974—1975, Érd-Elvira)

Treatment	Year of examination	Number per graft of		Effect of irrigation in 1971—1974 on girth at 50 cm above the ground (cm)
		inflorescences	flowers	
Inter-row cultivated, non-irrigated	1974	268	894	25.5
	1975	302	1027	—
Inter-row cultivated, irrigated	1974	373	1254	26.5
	1975	421	1474	—
Inter-row grassed, non-irrigated	1974	198	646	21.5
	1975	211	694	—
Inter-row grassed, irrigated	1974	382	1212	24.4
	1975	434	1394	—

As a response to irrigation carried out between 1971 and 1974 the girth at a height of 50 cm above the soil surface was greatest (26.6 cm) in the inter-row cultivated irrigated treatment, and smallest (21.5 cm) in that sown with grass between the rows and left unirrigated. In both treatments irrigation resulted in a larger trunk diameter.

The effect of irrigation on the beginning and duration of flowering. Flowering began 1—2 days earlier in the non-irrigated treatments. In the inter-row cultivated, non-irrigated treatment the main period of flowering (mass opening of flowers) in 1974 began a day earlier and lasted two days. In the inter-row cultivated, irrigated treatment, on the other hand, the engthe of the main period of flowering was three days. The same tendency was found in the lrrigated and non-irrigated treatments sown with grass between the rows. In 1975 mass blossoming in the inter-row cultivated, non-irrigated treatment began earlier and took a day less than

Table 2

Effects of irrigation and temperature on the daily caourse of (1974

Treatment	Dehiscent anthers (%)						
	5 April						
	6 a.m.	7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12
Inter-row cultivated, non-irrigated	—	—1.5	1.7	3.1	5.8	10.1	18.1
Inter-row cultivated, irrigated	—	1.0	1.5	2.4	6.2	10.0	17.2
Inter-row grassed non-irrigated	—	1.1	2.3	4.5	7.2	13.8	25.5
Inter-row grassed irrigated	—	2.0	2.1	6.8	9.9	10.6	17.3
Air temperature (°C)	1.8	—	8.5	—	13.0	—	15.2
Relative humidity (%)	88	—	57	—	42	—	31

in the irrigated treatment, where the main period of flowering was four days. A similar tendency was observed with the grass-sown treatments.

Comparing the treatments we find that the main period of flowering began earliest in the inter-row grassed, non-irrigated treatment, then in the inter-row cultivated non-irrigated treatment, followed by the inter-row grassed irrigated and inter-row cultivated irrigated treatments. The trees responded to irrigation with a 1—2 days longer period of mass blossoming compared to the non-irrigated treatments.

The effect of irrigation on the daily course of flower opening. The daily course of flower opening is shown in Figs 1—2. From the figures it is clear that flower opening was a continuous process throughout the day. Flower opening reached a maximum between 8 and 10 a.m. in each treatment. At 4 p.m. a lower peak was observed in both years. The morning maximum required a temperature of 10—13.5°C at crown height. The afternoon maximum of flower opening was much lower than that in the morning. The maximum values were attained at 12.6—13°C. The influence of irrigation on the daily course of flower opening was obvious in 1974. In the inter-row cultivated, non-irrigated treatment 94.5% of the marked flowers opened on 6th April, while in the inter-row cultivated irrigated treatment only 82.5% opened on this day. The maximum number of flowers opening per hour was 10% higher in the non-irrigated treatment than in the irrigated one.

In the non-irrigated treatment sown with grass between the rows 100% of the marked flowers opened on the day of examination, while in the irrigated treatment the corresponding value was 86.5%. The maximum number of flowers opening per hour was highest at 9 a.m., being 50.0% in the non-irrigated, and 32.5% in the irrigated treatment.

The effect of irrigation can be followed even better if the maximum values for the daily course of flowering are summarized. For example, in 1974 64% of the total number of flowers opening in a day opened between 8 and 10 a.m. in the inter-row grassed irrigated treatment and in the non-irrigated treatment 80%.

The tables show that the dehiscence of the anthers was continuous throughout the day. Dehiscence between 8 and 10 a.m. was of minor extent, and intensive from 11 a.m. to 2 p.m. In the irrigated treatments the maxima were lower than in the non-irrigated ones. Anther dehiscence took longer in the irrigated treatments. In 1974, for example, the dehiscence of the anthers took ten hours in the inter-row grassed non-irrigated treatment, and thirty

anther dehiscence in the sour cherry variety "Érdi bőtermő"
Érd-Elvira)

Dehiscent anthers (%)											Time required for the anthers to dehiscent (hours)
6 April											
1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	5 p.m.—7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12	
18.3	14.2	10.1	5.3	2.1	4.3	0.9	1.1	1.5	0.9		29
18.1	13.2	8.4	4.9	2.2	3.1	1.5	1.7	2.4	5.2	1.0	30
21.7	16.3	6.4	1.1								10
17.9	12.2	7.3	4.5	2.0	2.3	1.4	1.1	1.0	0.9	0.7	30
—	16.3	—	16.1	—	—	10.2	—	13.1	—	15.2	
—	29	—	29.5	—	—	70	—	43	—	35	

hours in the irrigated treatment. In 1975 dehiscence lasted for 56 hours in the inter-row grassed non-irrigated treatment. Anther dehiscence required less time in 1974 because the daily temperature maxima were higher (16—18°C), and took longer in 1975 when they ranged from 11 to 16.5°C. The dehiscence of anthers began at a temperature of 4.5—5°C.

Irrigation also exercised an influence on the viability of the pistil. In both years the functioning capacity of the pistil lasted 1—2 days longer in the irrigated treatments. This period was shortest (2—3 days) in the inter-row grassed non-irrigated treatment, followed by the inter-row cultivated non-irrigated one (3—6 days), while it was longer in the inter-row grassed irrigated (4—6 days) and inter-row cultivated irrigated treatments (4—7 days).

From the observations the following conclusions can be drawn: 1) The beginning, duration and daily course of flowering were primarily influenced by the trend of temperature. Irrigation exerted its effect through the water regime of the trees. 2) The effect of irrigation on the extent of flowering and the opening of the flowers manifested itself primarily through the condition of the trees. Under irrigated conditions the number of inflorescences and flowers was larger, and the generative organs maintained their functions for a longer time. 3) In irrigated treatments the longer period of pistil viability and pollen distribution increases the reliability of pollination. 4) Modifications in the flowering time under the influence of irrigation should be reckoned with when choosing the pollinating varieties.

*

Prepared at the University of Horticulture, Department of Plant Genetics and Breeding and at the Horticultural Research Institute, Budapest.

J. Nyéki, Z. Ifjú, I. Gergely

Table 3

Effects of irrigation and temperature on the daily course of anther dehiscence in the sour cherry variety "Érdi bőtermő"
(1975, Érd-Elvira)

Treatment	Dehiscent anthers (%)											
	17 April											
	6	7	8	9	10	11	12 noon	1	2	3	4	5
	a.m.							p.m.				
Inter-row cultivated, non-irrigated	—	1.0	1.1	2.1	3.5	11.1	13.2	12.1	10.2	6.2	3.2	1.2
Inter-row cultivated, irrigated	—	0.4	0.9	1.1	2.2	6.7	6.9	6.2	5.1	3.2	2.1	0.9
Inter-row grassed, non-irrigated	—	1.5	1.6	2.5	4.1	14.2	15.3	15.5	11.4	7.3	4.4	2.3
Inter-row grassed, irrigated	—	0.8	1.1	1.5	2.4	7.3	7.5	7.2	5.6	4.2	2.5	1.0
Air temperature (°C)	8.5	—	10.9	—	12.2	—	13.1	—	13.2	—	12.0	—
Relative humidity (%)	85	—	75	—	63	—	56	—	55	—	54	-

Treatment	Dehiscent anthers (%) 18 April										
	5 p.m.—7 a.m.	8	9	10	11	12 noon	1	2	3	4	5
		a.m.					p.m.				
Inter-row cultivated, non-irrigated	2.2	0.6	1.1	1.7	8.1	7.2	5.5	4.8	2.1	1.2	1.1
Inter-row cultivated, irrigated	1.9	0.4	0.8	1.2	7.1	7.4	5.3	3.2	1.2	0.9	0.3
Inter-grow grassed, non-irrigated	3.5	1.2	2.9	3.1	8.2						
Inter-grow grassed, irrigated	2.3	0.8	1.1	2.1	7.4	7.9	6.1	3.0	1.5	1.0	0.9
Air temperature (°C)	—	5.0	—	8.2		—11.0	—	13.0	—	13.2	—
Relative humidity (%)	—	92	—	90	—	73	—	55	—	45	—

Treatment	Dehiscent anthers (%) 19 April								Time required for anthers to dehiscent (hours)
	5 p.m.—7 a.m.	8	9	10	11	12 noon	1	2	
		a.m.					p.m.		
Inter-row cultivated, non-irrigated	1.2	0.3							50
Inter-row cultivated, irrigated	1.1	0.4	1.1	2.2	10.2	9.3	9.4	0.9	56
Inter-grow grassed, non-irrigated									29
Inter-grow grassed, irrigated	2.2	0.5	1.2	2.5	8.2	5.2	4.3	0.8	56
Air temperature (°C)	—	4.8	—	9.8	—	11.2	—	12.8	
Relative humidity (%)	—	92	—	85	—	70	—	45	

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GENETIC VARIABILITY AND INTERRELATIONSHIPS OF SOME QUANTITATIVE CHARACTERS IN GEOGRAPHICAL GROUPS OF EXOTIC LINSEED (LINUM USITATISSIMUM L.) GERM PLASM

Linseed is an important oilseed crop in India. It provides the major raw material for the varnish and linoleum industries, because of the drying property of its oil. Besides this it has been given new impetus since its oil has been recognized as an important component in road construction, particularly in hilly areas (WALSH 1965). However, the yield of the crop is markedly low in this country in comparison to other linseed growing countries. One of the

basic factors for low yield is the lack of genetic variability among the indigenous varieties which hitherto have been used in breeding programmes for yield improvement. In such a situation, several workers (KONZAK—DIETZ 1969, FRANKEL—BENNETT 1970, KRULL—BORLAUG 1970, CREECH—REITZ 1971) have suggested the evaluation of exotic germ plasm to develop a population with a broad genetic base. With this objective, a programme was formulated to investigate the genetic variability of various quantitative characters directly or indirectly concerned with seed yield in regional germ plasm resources and to bring out meaningful associations between them. The results of such a study are reported in the present paper.

The experimental material consisted of 145 cultures representing different geographical regions (12 from Afghanistan, 20 each from Argentina, Australia, Canada, Germany and the USA, 10 from France, 8 from Sweden and 15 from the USSR). They were grown in a randomized complete block design with two replications during the winter season of 1968—69 at Delhi. Each plot included three rows of 3 m length each. The rows were spaced 30 cm apart and the spacing of plants within a row was 15 cm. Five competitive plants were randomly selected from the middle row of each plot to record observations on the following characters: boll number, tiller number, 1000-seed weight (g), days to first and 50% flowering, and seed yield (g). Plot means were used to calculate the range of variation, the mean, and the phenotypic and genotypic coefficients of variation (PCV and GCV) for each character in each geographical group and also in the total collection. Phenotypic correlation coefficients between various characters were derived from phenotypic variance-covariances according to the formula given by AL-JIBOURI *et al.* (1958).

The results of analysis of variance for 145 cultures are presented in Table 1. Cultures in the total collection differed significantly from each other with respect to all the characters studied. When the total collection was further partitioned into the respective geographical groups, significant differences were again observed for these characters in each group, except for boll and tiller number in the French and German groups. Furthermore, the groups differed significantly among themselves with respect to all the characters, suggesting that the geographical groups were also genetically distinct, forming germ plasm pools.

Table 1

Analysis of variance for various characters in 145 cultures

Source of variation	d.f.	MSS					
		Boll number	Tiller number	1000-seed weight	Days to first flowering	Days to 50% flowering	Seed yield
Replications	1	66,455.50**	74.01**	1.13	14.10	39.80**	34.83**
Cultures	144	5,787.63**	8.82**	2.88**	70.64**	91.99**	8.15**
Afghan	11	5,553.86**	6.58**	0.79*	54.68**	40.86**	4.86**
Argentine	19	8,309.34**	7.31**	1.52**	63.76**	32.13**	10.22**
Australian	19	3,765.08**	7.36**	3.78**	24.92**	45.47**	4.22**
Canadian	19	3,924.43**	6.31**	0.89**	41.68**	55.24**	9.62**
French	9	1,636.01	4.20	2.11**	65.69**	82.20**	3.78**
German	19	1,612.96	3.13	0.58**	47.45**	65.63**	2.71**
Swedish	7	2,981.71*	9.34**	1.54**	44.59**	83.99**	6.80**
USA	19	4,226.54**	5.94**	5.63**	90.13**	100.43**	7.12**
USSR	14	3,559.94**	6.77**	5.12**	40.85**	33.99**	7.04**
Between geographical groups	8	33,995.29**	51.22**	8.62**	375.57**	662.28**	1.34
Error	289	1,616.82	3.03	0.42	6.91	11.23	1.80

* Significant at 5% ** Significant at 1%

Table 2

Range of variation, mean, phenotypic and genotypic variance and coefficients of variation for various characters

Country	Range of variation	Mean	Phenotypic variance	Genotypic variance	PCV	GCV
Boll number						
Afghanistan	80.0—246.5	155.8	3585.34	1968.53	38.44	28.49
Argentina	99.5—327.5	206.2	4963.08	3346.27	34.15	28.05
Australia	93.5—303.0	166.6	2690.95	1074.14	31.14	19.67
Canada	62.0—202.0	131.2	2770.62	1153.81	40.13	25.90
France	109.0—205.0	141.8	1626.41	9.58	28.43	2.18
Germany	58.5—151.0	99.9	1614.89	—19.2	40.21	—
Sweden	79.5—189.0	121.38	2921.67	1304.86	44.53	29.76
USA	60.0—221.5	124.2	2299.26	682.45	38.61	21.03
USSR	80.0—212.5	142.9	2588.19	971.38	35.59	21.81
Total collection	58.5—327.5	144.4	3702.39	2085.85	42.08	37.60
Tiller number						
Afghanistan	7.5— 13.5	9.4	4.81	1.78	23.30	14.17
Argentina	4.5— 10.5	7.1	5.17	2.14	31.91	20.53
Australia	3.0— 11.5	6.6	5.20	2.17	34.80	22.47
Canada	3.0— 9.5	6.4	4.67	1.64	33.50	19.86
France	3.5— 9.0	6.4	3.62	0.58	29.71	11.95
Germany	1.5— 6.5	4.5	3.08	0.05	39.22	5.00
Sweden	3.5— 8.5	6.4	4.48	1.45	33.12	18.86
USA	2.0— 10.5	6.2	6.18	3.15	40.11	28.64
USSR	3.5— 9.5	6.3	4.90	1.87	35.14	21.17
Total collection	1.5— 13.5	6.5	5.92	2.89	37.60	26.28
1000-seed weight						
Afghanistan	3.8— 6.2	4.5	0.62	0.17	17.41	9.16
Argentina	4.5— 8.8	6.0	0.89	0.54	16.62	12.25
Australia	4.0— 9.5	6.6	3.78	1.66	22.17	19.67
Canada	4.0— 6.5	5.3	0.67	0.22	15.53	8.95
France	4.8— 8.0	6.1	1.28	0.83	18.63	15.01
Germany	4.5— 6.2	5.2	0.51	0.06	13.70	4.84
Sweden	4.2— 9.0	5.6	3.04	2.59	31.86	28.94
USA	3.2— 6.2	4.3	0.99	0.54	20.99	15.54
USSR	3.5— 9.8	5.5	2.78	2.33	30.42	27.85
Total collection	3.2— 9.8	5.5	1.63	1.14	23.30	19.78
Days to first flowering						
Afghanistan	89.0—106.0	99.8	30.79	23.89	5.56	4.90
Argentina	89.0—111.0	98.8	35.33	28.43	6.01	5.39
Australia	82.0— 97.5	88.7	15.91	9.01	4.50	3.38
Canada	84.5—102.0	92.1	24.29	17.39	5.36	4.53
France	89.0—103.0	94.8	36.30	29.40	6.36	5.72
Germany	82.5—101.5	91.9	27.18	20.28	5.67	4.90
Sweden	88.0—107.0	94.8	48.52	41.62	7.35	6.80
USA	87.5—103.0	91.7	25.75	18.85	5.53	4.74
USSR	83.5—1015.	90.7	23.87	16.97	5.39	4.54
Total collection	82.0—111.0	93.3	38.77	31.86	6.67	6.05
Days to 50% flowering						
Afghanistan	98.5—113.5	109.5	26.04	14.82	4.66	3.52
Argentina	99.5—113.0	109.0	21.67	10.45	4.26	2.96
Australia	88.5—107.5	96.6	28.34	17.12	5.51	4.29
Canada	92.0—110.5	97.7	33.23	22.01	5.78	4.70
France	94.6—112.5	102.1	47.71	36.49	6.77	5.92

Country	Range of variation	Mean	Phenotypic variance	Genotypic variance	PCV	GCV
Germany	93.5—112.5	100.4	38.38	27.16	6.17	5.19
Sweden	94.5—113.0	101.2	55.82	44.60	7.38	6.60
USA	90.5—112.0	98.6	47.61	36.39	7.00	6.12
USSR	94.0—110.0	104.4	22.64	11.38	4.73	3.36
Total collection	88.5—113.5	101.7	51.60	40.38	7.07	6.25
Seed yield						
Afghanistan	2.4— 7.4	3.8	3.28	1.48	47.48	31.90
Argentina	3.3— 11.2	6.9	6.01	4.21	35.72	29.90
Australia	2.4— 8.2	5.5	3.01	1.21	31.68	20.09
Canada	2.5— 9.3	5.5	5.72	3.91	43.35	35.88
France	3.7— 8.0	5.5	2.79	0.00	30.41	18.12
Germany	2.2— 5.8	3.7	2.27	0.46	40.11	18.03
Sweden	2.7— 8.1	4.4	4.46	2.66	48.07	37.12
USA	1.4— 8.7	4.1	4.30	2.50	50.82	38.75
USSR	2.8— 9.0	5.2	4.42	2.62	40.46	31.16
Total collection	1.4— 11.2	5.0	4.98	3.17	44.47	35.51

The range of variation, the mean, the phenotypic and genotypic variance and the coefficients of variation for six characters are given in Table 2.

Boll number showed a high range of variation, varying from 58.5 for lines from Germany to 327.5 for lines from Argentina. The mean value of the individual group varied from 99.9 for the German lines to 206.2 for the Argentine lines with a moderate GCV (28.05%). The German lines were highly susceptible to the environment, with the result that negative genotypic variance was registered, giving rise to a negative value of GCV, which is theoretically impossible and can be best considered as a zero value.

Tiller number ranged from 1.5 for the German lines to 13.5 for the Afghan lines. With respect to the individual group mean, the German lines possessed a minimum number of tillers (4.5) and the Afghan lines a maximum number (9.4). Although the Afghan lines showed maximum tillering, their GCV (14.17%) was relatively low. Next to the Afghan lines, Argentine and US lines had a high tiller number with a moderately high GCV (20.53 and 28.64% respectively).

The range of variation for 1000-sede weight varied from 3.2 for the US lines to 9.8 for the Russian lines, and the group mean ranged from 4.8 for the USA lines to 6.6 for the Australian lines. The GCV, in general, for all the groups was relatively low, the lowest being for the German lines (4.84%) and the highest for the Swedish lines (28.94%). The Australian lines, whose mean was comparatively high (6.6), showed a moderate GCV (19.67%).

Days to first and 50% flowering had a low GCV. Days to first flowering ranged from 82.0 for lines from Australia to 111.0 for lines from Argentina. The minimum for days to 50% flowering was again recorded in Australian lines (88.5) and the maximum in Afghan lines (113.5). The earliest flowering types, on average, thus came from the Australian group and the late flowering types from the Afghan group.

A good range of variation was observed for seed yield in the present material, varying from 1.4 for the US lines to 11.2 for the Argentine lines. The maximum average seed yield on a group basis was observed in the Argentine types (6.9), with 29.90% GCV.

In most breeding programmes, improvement is sought in a number of characters and the progress achieved will depend upon the nature and extent of the relationships between the variables. The relationships between the various characters studied were estimated in terms of phenotypic correlation coefficients, which are presented in Table 3.

Table 3

Phenotypic correlation coefficients between various characters within geographical groups and total collection

Character combination \ Country	Afghanistan	Argentina	Australia	Canada	France	Germany	Sweden	USA	USSR	Total collection
Seed yield vs.										
boll number	0.622*	0.747**	0.345	0.840**	0.248	0.687**	0.718**	0.498*	0.498	0.686**
tiller number	0.051	0.404*	0.372	0.572**	0.505	0.506*	0.082	0.641**	0.253	0.376
1000-seed weight	0.270	0.103	0.565**	0.204	0.557	0.141	0.724*	0.433*	0.360	0.418**
days to first flowering	−0.363	−0.059	0.033	−0.233	−0.320	0.100	0.022	0.267	−0.154	0.009
days to 50% flowering	−0.041	−0.023	0.024	−0.107	−0.340	−0.193	0.011	0.261	−0.175	0.044
Boll number vs.										
tiller number	0.309	0.468*	0.637	0.565**	0.311	0.481*	0.325	0.570**	0.676**	0.530**
1000-seed weight	−0.199	0.068	−0.098	0.047	0.104	−0.115	0.626	0.130	−0.151	0.152
days to first flowering	−0.164	0.168	−0.081	−0.103	−0.091	−0.034	−0.011	0.109	0.358	0.163**
days to 50% flowering	−0.239	0.100	−0.262	0.054	−0.206	−0.099	−0.025	0.117	0.291	0.160**
Tiller number vs.										
1000-seed weight	−0.199	−0.102	−0.013	0.026	0.150	0.042	0.082	0.453*	−0.313	−0.221*
days to first flowering	0.221	−0.110	−0.237	0.215	0.097	0.281	0.400	0.481*	0.362	0.290**
days to 50% flowering	0.236	−0.071	−0.487*	0.346	0.171	0.118	0.431	0.339	0.393	0.278**
1000-seed weight vs.										
days to first flowering	0.081	−0.194	0.364	−0.149	0.469	−0.069	−0.173	0.274	−0.296	−0.107
days to 50% flowering	−0.042	−0.292	0.371	−0.069	−0.531	−0.029	−0.125	0.217	−0.285	−0.072
Days to first flowering vs.										
days to 50% flowering	0.823**	0.622**	0.713**	0.809**	0.842**	0.774**	0.914**	0.791**	0.751**	0.831**

* Significant at 5%. ** Significant at 1%.

A perusal of the table shows that important attributes of the seed yield were boll and till number, and 1000-seed weight in the total collection. These influenced the seed yield positively and were in general agreement with previous reports by SAXENA—ASTHANA (1962), PATHAK—BAJPAI (1964), BADWAL *et al.* (1970) and VIJAYAKUMAR—RAO (1974). Significant relationships of the seed yield with boll and tiller number and 1000-seed weight were established in most of the groups. However, the relationships changed in extent in the respective groups. The correlation between seed yield and boll number was positive and significant in lines from Afghanistan, Argentina, Canada, Germany, Sweden and USA. The correlation of seed yield with tiller number was positive in all the groups, but significant only in the Argentine, German, Canadian and US groups. 1000-seed weight was positively and significantly correlated with seed yield in the Australian, Swedish and US groups only. Flowering did not influence the seed yield.

Significant correlations were also marked in some of the combinations of other characters.

These results demonstrate that an abundance of genetic variability existed in germ plasm resources from different regions for boll and tiller number and also for 1000-seed weight, which primarily determined the seed yield. Argentine lines offered an opportunity for improving boll and tiller number and Australian lines for improving seed size. Through hybridization, Indian lines can be improved for seed yield and its component characters by incorporating genes from these germ plasm pools. Moreover, the regional germ plasm pools, which have diverged due to natural and/or human selection, can be recombined to produce even more variability and to reconstruct better plant types with an increase in their physiological efficiency.

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Prepared at the Indian Agricultural Research Institute, New Delhi.

S. CHANDRA, P. O. MAKHIJA

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THE EFFECT OF GA_3 and CCC ON THE LEVELS OF ENDOGENOUS PLANT HORMONES. II. THE EFFECT OF GA_3 AND CCC ON ENDOGENOUS GIBBERELLIN LEVELS IN DIFFERENT WHEAT VARIATIES

In the literature there are a number of publications (ZEEVAART 1966, DALE—FELIPPE 1968) with respect to the effect of CCC on the endogenous gibberellin level in plants. Yet no special attention has been paid in this respect to the diversity between variaties, nor has this diversity been considered when studying the changes in the endogenous gibberellin level in response to treatment with GA. In view of this, the present work was conducted to deal with a species rarely studied in this field, namely *Triticum vulgare* (wheat). This investigation aimed to show how the type of variety could modify the effect of either GA_3 or CCC on endogenous gibberellin levels in wheat seedlings.

The present work was conducted during 1973 in a controlled climate chamber in the Institute of Plant Physiology, Aarhus University, Aarhus, Denmark. Four wheat varieties were used: Progress, Solo, Drabant and Toyota. The seedlings of each variety were sprayed on the 20th day after sowing with either gibberellic acid (GA_3) in 10, 50 and 100 mg/l doses or with 2 (chloreothyl) trimethylammonium chloride (CCC) in 100, 500 and 1000 mg/l doses. Sampling, using fresh material taken at random from the shoot system, was conducted on the 41st day after sowing. The plants were treated for the extraction of hormones with 80% redistilled cold methanol, and an aliquot equivalent to 5 g fresh weight was loaded across the start lines of 3.5 cm wide strips of Whatman No. 3 chromatography paper. The chromatograms were developed in tanks lined with filter paper using a solvent composed of isopropanol : ammonia : water (10 : 1 : 1, v/v) and were air-dried. In order to test the presence of gibberellins, the GA_3 control strip was immersed in a solution of concentrated sulphuric acid and ethyl alcohol (95 : 5 v/v) and warmed for 1—2 minutes in an oven and then examined under a UV lamp (after RUSSELL—KIMMINS 1971). For the determination of endogenous gibberellins the lettuce hypocotyl assay was used (FRANKLAND—WAREING 1960). The results were statistically analysed according to TUKEY (1953). The data obtained were represented as histograms. The details of the techniques adopted for carrying out the experiment, fertilization, sampling and extraction of hormones were described in a previous paper (RAAFAT *et al.* 1978).

1. Gibberellin levels in untreated wheat seedlings. From Fig. 1 it could be shown that the level of endogenous gibberellins varied according to the type of variety used. This level appeared to be the highest in Progress and Drabant, particularly in the former, whereas it was the lowest in Toyota. The activities of endogenous gibberellins were located at R_{fs} 0.0—0.1 and 0.3—0.6; 0.5—0.6; 0.2—0.5; and 0.4—0.5 for Progress, Solo, Drabant and Toyota respectively.

2. Gibberellin levels in wheat seedlings treated with gibberellic acid. From Figs 1 and 2 it appeared that the application of any of the GA_3 concentrations distinctly raised the endogenous level of gibberellins (compared with the controls) whatever type of variety was used. The magnitude of this rise in all the varieties appeared to be more pronounced as the dose of applied GA_3 was increased from 10 mg/l (the lowest level) to 50 mg/l (the medium level). Nevertheless, the response exhibited after a further rise in the GA_3 concentration applied depended upon the kind of variety. This further increase in the GA_3 dose only raised the level of endogenous gibberellins in the case of Progress; in the other three varieties the reverse was found to be true.

The R_{fs} values for gibberellin activities in seedlings treated with the low, medium and high GA_3 concentrations were in the order: 0.3—0.6; 0.0—0.1 and 0.2—0.7; and 0.0—0.7 for Progress; 0.0—0.1 and 0.3—0.6; 0.0—0.1 and 0.2—0.8; and 0.0—0.1 and 0.2—0.7 for Solo; 0.2—0.6; 0.0—0.1 and 0.2—0.7; and 0.0—0.6 for Drabant; 0.3—0.6; 0.0—0.7; and 0.2—0.7 for Toyota.

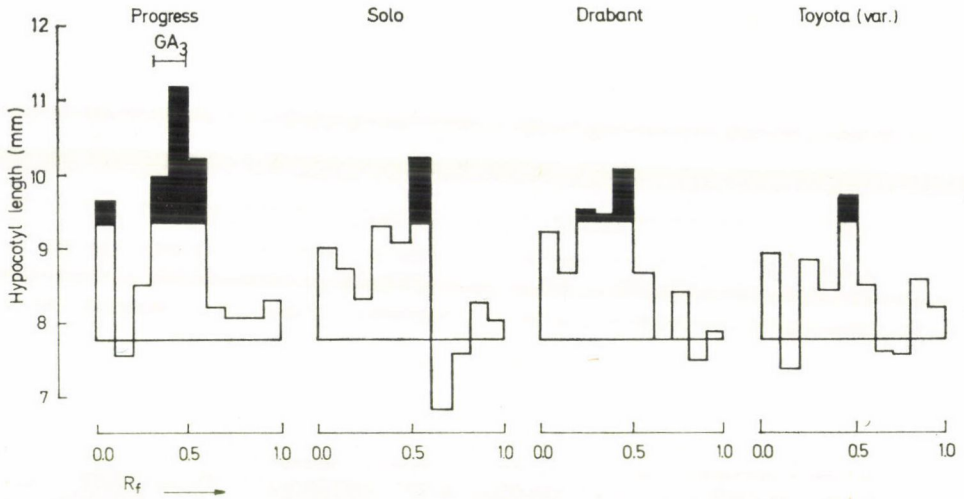

Fig. 1. Distribution of gibberellin activity on chromatograms developed with isopropanol : ammonia : water (10 : 1 : 1 v/v) for extracts of four varieties of wheat seedlings. The lettuce hypocotyl assay was used for estimation. The location of a marker spot for GA_3 is shown by a horizontal bar. Shaded parts represent biological activity at a 1% level of significance

3. Gibberellin levels in wheat seedlings treated with CCC. From Figs 1 and 3 it could be indicated that in Progress, Drabant and Toyota each dose of CCC was so effective in decreasing the activity of endogenous GA, compared with the untreated seedlings, that it was beyond the significant level in each case. Even in Solo, an analogous picture was noted with regard to the low (100 mg/l) and high (1000 mg/l) CCC concentrations. On the other hand, a certain significant level for the endogenous GA activity was detected in Solo when the medium CCC dose was applied (at R_f 0.4—0.6). This level was of a magnitude similar to that in the controls.

The present investigation showed certain differences between the varieties used with respect to the endogenous gibberellin level in the untreated seedlings. This type of observation corresponds with KÖHLER (1970), RADLEY (1970) and GOTÔ—ESASHI (1973). According to the data obtained, it was also indicated that all the varieties showed highly significant amounts of gibberellin activity after GA_3 application. This observation corresponds with that of EL-ANTABLY (1970). It was particularly noted that in this study the type of variety appeared to have a role in determining the magnitude of the positive response shown by endogenous gibberellin activity to the application of a given dose of GA_3.

On the other hand, the present investigation showed that CCC application lowered the endogenous GA activity in the different varieties. This type of observation is in harmony with the general trend reported in the literature (ZEEVAART 1966, DALE—FELIPPE 1968).

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Prepared at the Department of Plant Pathology and Agricultural Botany, Faculty of Agriculture, Ain-Shams University, Shobra El-Kheima.

A. I. GABR, A. RAAFAT, G. FARRAG

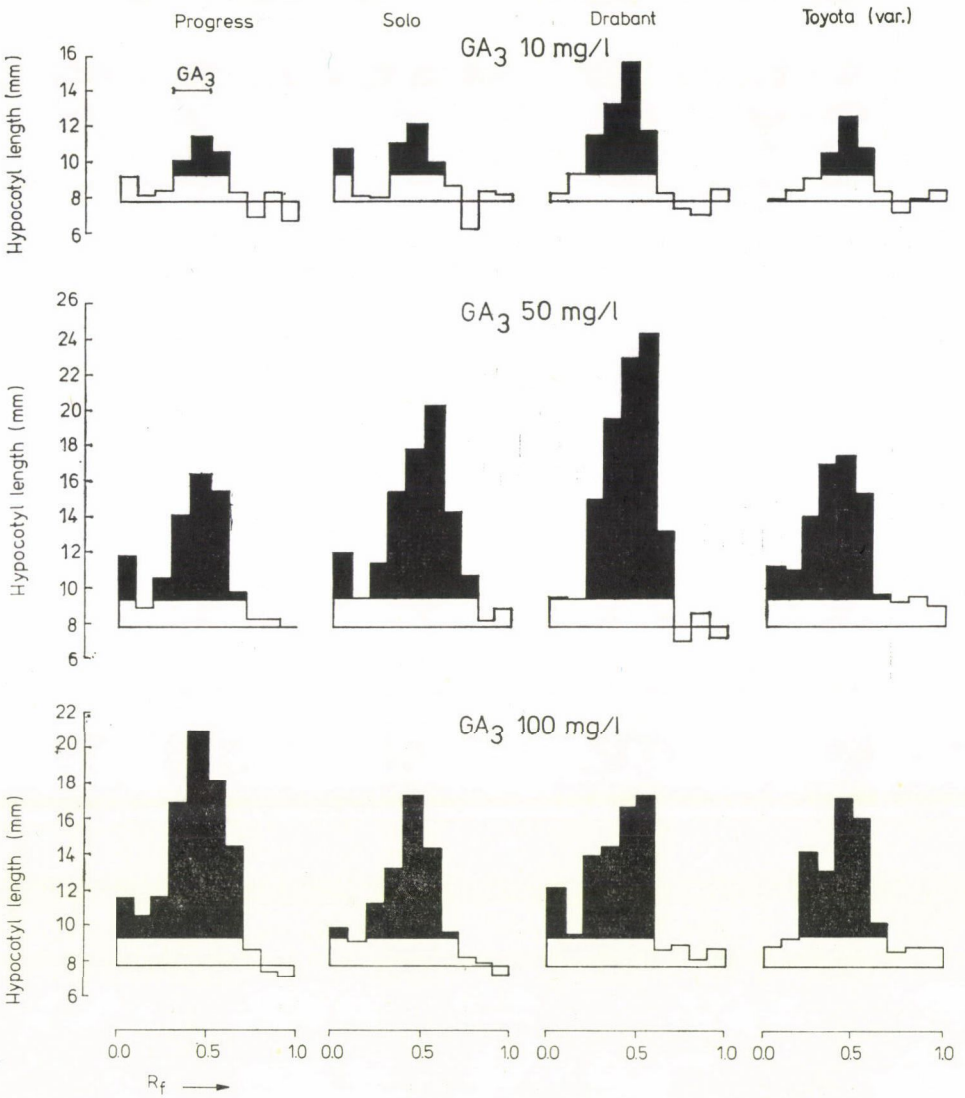

Fig. 2. Distribution of endogenous gibberellins on chromatograms of extracts of four varieties of wheat seedlings treated with three GA_3 concentrations, the chromatograms being developed with isopropanol : ammonia : water (10 : 1 : 1 v/v). The lettuce hypocotyl assay was used for estimation. The location of a marker spot for GA_3 is shown by a horizontal bar. Shaded parts represent biological activity at a 1% level of significance

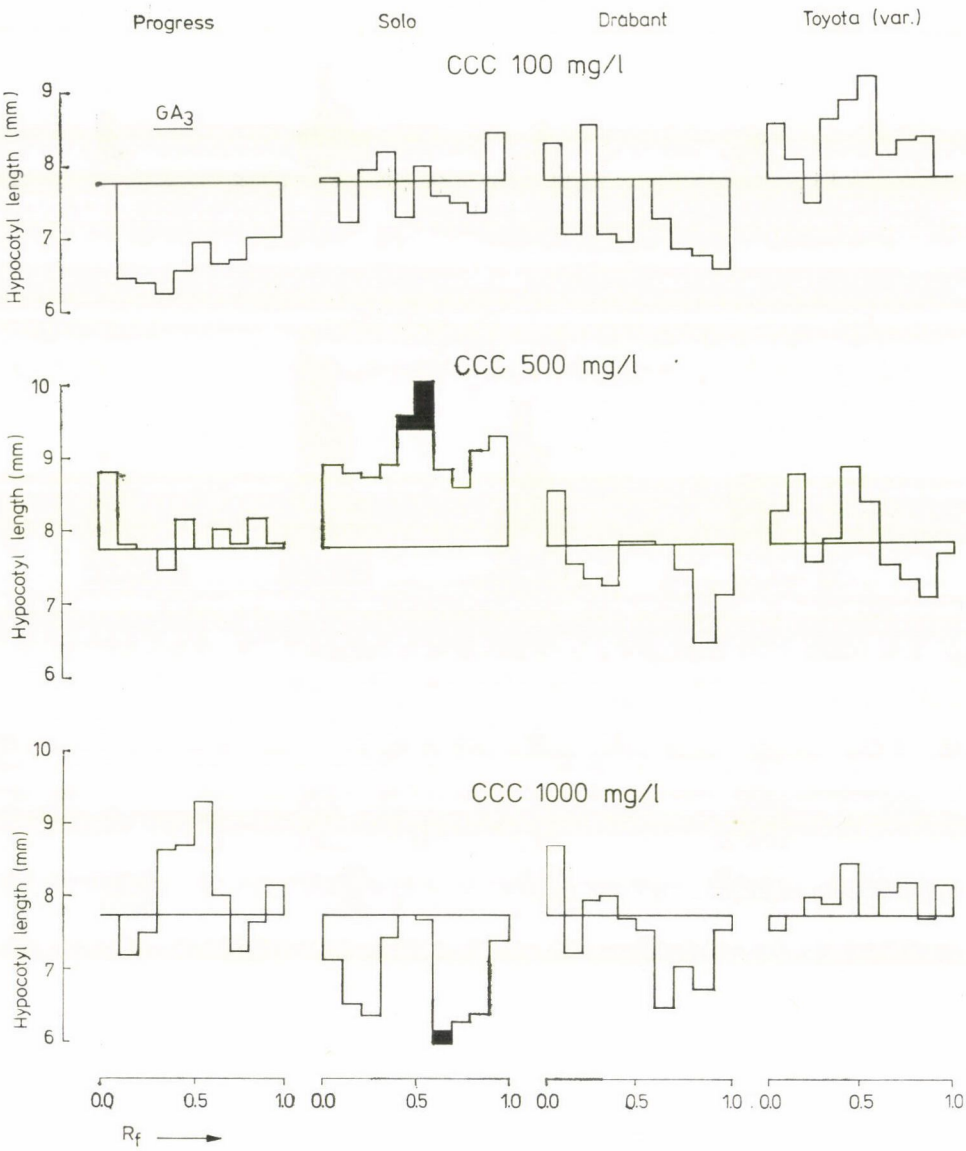

Fig. 3. **Distribution of endogenous gibberellins on chromatograms of extracts of four varieties of wheat seedlings treated with three CCC concentrations, the chromatograms being developed with isopropanol : ammonia : water (10 : 1 : 1 v/v). The lettuce hypocotyl assay was used for estimation. The location of a marker spot for GA_3 is shown by a horizontal bar. Shaded parts represent biological activity at a 1% level of significance**

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BIOPOLYMER — METAL COMPLEX SYSTEMS. VII.

STUDY OF ION EXCHANGE AND REDOX CAPACITY OF PEAT HUMIC SUBSTANCES

Interactions between humic substances and cations have been treated both as physical phenomena: adsorption, peptization or coagulation, and as chemical reactions: ion exchange, formation of chelate type inclusion complexes or salt formation (BUZÁGH 1951, VAN DIJK 1971, MORTENSEN 1963, ONG—BISQUE 1967, SZALAY—SZILÁGYI 1967, WILDENHAIN 1969). In Hungary the Szalay School (SZALAY—ALMÁSSY 1956, SZALAY—SZILÁGYI 1967), applying Langmuir's adsorption isotherms to these systems, represented the physical aspect. In general, the adsorption of cations (except for iron(III) and chromium(III) ions (SZABÓ 1958) on humic acids could be well described by the Langmuir formula (SZABÓ 1958). A member of this school also attempted an interpretation of the natural enrichment of trace metals by Szalay's theory of cation and anion adsorption (SZILÁGYI 1970). Most recently Szalay suggested, with reference to Mössbauer effect measurements, that iron(II) ions are adsorbed on humic acids in the form of hydrated iron(II) ions. BUZÁGH (1951) and other researchers (DESAI *et al.* 1970, VAN DIJK 1971), on the other hand, presumed the presence of mainly chemical reactions: chemisorption, topochemical processes, ion exchange and chelate complex formation. The assumption that, in addition to physical adsorption, the formation of chemical bonds must be considered is supported by the following experimental facts:

1. During electrophoresis, metal ions in aqueous solution with an excess amount of humic acids show two different behaviours (KLEIST 1963). In maintaining their positive charge, alkali and alkali earth metal ions migrate to the cathode and are detectable by emission spectroscopy. On the other hand, univalent ions (e.g. silver), bivalent 3d-transition metal ions (e.g. iron(II), cobalt(II) and copper(II) ions), meta-metal ions (e.g. lead(II) and thallium(III) ions) and finally, trivalent cations (e.g. aluminium(III) ions) migrate to the anode (KLÖCKING *et al.* 1967).

Paper electrophoresis studies of iron(III) and aluminium(III) complexes of fulvic acids at pH = 7—10 suggest the formation of complexes with a negative charge and much higher mobility than found for the corresponding humic acid complexes (SCHNITZER—KHAN 1972). During paper electrophoresis of brown humic acids containing germanium dioxide, some migrated to the anode while others remained at the starting point in the form of uncharged complexes (WILDENHAIN 1969).

Radiochromatographic measurements with ^{59}Fe isotope performed in the presence of an aqueous solution of fulvic acid showed the migration rate of iron(III) fulvate complexes to be higher than that of the free iron(III) aquoion (KLEIST 1964, KLÖCKING *et al.* 1967).

2. The reaction of humic acids with metal ions in aqueous solution with partial displacement of the hydrogen ion and the formation of inner sphere metal humates (SCHNITZER—KHAN 1972) can be described by the following scheme:

$$Me\,(H_2O)_q^{n+} + HU \rightarrow Me\,(H_2O)_{q-1}\,U^{n-1} + H_3O^+$$

$$Me\,(H_3O)_{q-1}\,U^{n-1} + HU \rightarrow Me\,(H_2O)_{q-2}\,U_2^{n-2} + H_3O^+$$

$$\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots$$

$$Me\,(H_2O)\,U_{q-1}^{n-q+1} + HU \rightarrow MeU_q^{n-q} + H_3O^+$$

where HU = humic acid and U = the humate anion. As can be seen, total ion exehange capacity is determined by the acid number, i.e. by the maximum number of exchangeable hydrogen ions. The concentration of hydrogen ions formed can be determined by potentiometry (POMMER—BREGER 1960a, 1960b) or by conductivity measurements (JOHNSON—HNOJEWYJ 1966). The relative, apparent equilibrium stability constants of the complexes formed can be determined by Job's continuous variation, by Schubert's ion-exchange methods or by means of metal ion selective membrane electrodes (SCHNITZER—KHAN 1972). The high values of total ion exchange capacity and relative, apparent complex stability constants point to the presence of chemical complex bonds.

3. Radiospectroscopic investigation, e.g. the EPR analysis of 3d-transition metal ions doped in peat humic acids, suggests the formation of inner sphere chelate complexes (LAKATOS *et al.* 1978), while in NQR studies, the linewidth broadening of the ^{85}Rb isotope in the presence of humic acid points to chemical bonds (LINDMAN—LINDQUIST 1969).

4. Our Mössbauer effect studies support the formation of high spin number inner sphere chelate type iron(II) complexes with water molecules in the first coordination sphere (LAKATOS *et al.* 1977).

5. The redox capacity of the humic substances allows the reduction of some transition metal anions and the bonding of the transition metal ions formed. This has been verified both by EPR measurements (LAKATOS *et al.* 1978) and by analytical chemical investigations (BOROMISSZA—VARGA 1972, SZIGÁGYI 1970).

6. In the DTG and DTA peaks of metal humates and fulvates, a shift towards lower degrees of temperature related to initial humic and fulvic acids can be observed (SCHNITZER—KHAN 1972).

The above observations support the assumption of mainly chemical bonds in addition to physical adsorption. Unfortunately, we have only a few data on the quantitative relationship between the two different types of bonds in soils. In the case of the cadmium(II) ion, for example this is reported to be 50% for each.

Our present paper concerns problems outlined in points 2 and 5.

The extraction, purification and analysis of the humic substances obtained from Keszthely lowland peat has been described earlier (LAKATOS *et al.* 1977). I-humic acid with a high protein content was extracted with sodium hydroxide and II-humic acid with a low protein content was obtained with sodium pyrophosphate from lowland peat (Keszthely, Hungary).

Protein free hydrolyzed humic acid was prepared by acid hydrolysis from I-humic acid. Humic acid extracted with sodium pyrophosphate, precipitated with hydrochloric acid and finally treated with ammonium hydroxide will be designated as III-humic acid.

For acid number determination, 0.1 g humic acid or fulvic acid was diluted in boiled, distilled water (25 cm^3 pH = 2.7) then titrated with 0.1 N sodium hydroxide. The change in pH values was recorded by a glass electrode pH meter, type Radelkis OP-205. The acid number values were derived from the titration curve.

The cation exchange capacity was determined by adding an aqueous solution of sodium humate to a given amount of excess 0.1 M metal salt (perchlorate or sulphate) (pH = 7). The mixture was then shaken vigorously for a period of 6 hours and separated by centrifugation. The pH value (the so-called final pH) of the supernatant was determined for each case and the amount of metal ions of the aliquot was determined by complexometry. The precipitate was freed from metal ion contamination by washing with distilled water and drying at 105—110°C .The precipitate was dissolved in a mixture of concentrated sulphuric acid (1 cm^3) and 30% hydrogen-hyperoxide (5 cm^3) by means of Schulek's digestion and the metal content was determined again by complexometry. In the case of metal fulvates and magnesium humates measurements were carried out in a 3 : 2 acetone to water mixture, in order to obtain precipitates in a quantitative amount.

The determination of cation exchange capacity at constant pH value was carried out using the following procedure: a 0.6% solution of humic acid (10 cm^3) was added to 1 M acetic acid-sodium acetate buffer (10 cm^3; pH = 2.7). Then, the aqueous solution of 0.1 M metal (II) acetate (10 cm^3) was mixed with a 1 M acetic acid-acetate buffer (10 cm^3) and the humic acid solution was added to the mixture dropwise. The system was then diluted to 100 cm^3 with a 1 M acetate buffer and allowed to stand overnight for complete precipitation. The precipitate was separated by centrifugation, freed from metal ions by washing and dried at 105—110°C. Part of the precipitate (0.05 g) was digested by Schulek's method and the metal ion and sodium ion content was determined by atomic absorption spectrometry. The hydrogen ion concentration of the carboxylic group was determined by the method used for calcium acetate measurements.

The ion exchange capacity for the two simultaneously present cations (iron and copper) was determined as follows: a 1% aqueous solution of humic acids (5 cm^3; pH = 4) was added dropwise to an excess amount (4 cm^3) of a mixture of 0.1 M iron(II) and copper(II) sulphates in aqueous solution in ratios of 1 : 1, 3 : 1 and 1 : 3. The system was allowed to stand overnight, and was then separated from the solution by means of a Sartorius membrane filter. The precipitate was washed three times with 10 cm^3 distilled water and the total amount of metal ions in the solution was determined by complexometry.

In an identical sample, by the addition of an excess amount of concentrated hydrogen peroxide, the iron(II) ion was oxidized quantitatively to iron(III) ion. After being boiled for 1 min. the hydrogen peroxide excess was decomposed. Iron(III) ion was masked by the addition of approx. 2 g sodium fluoride and the excess of copper(II) ion was titrated with 0.02 M EDTA in the presence of a xylenol orange indicator after the addition of acetate buffer at pH = 5. The precipitate was freed from metal contaminations by washing, then dried at 105—110°C and measured. After being dissolved by Schulek's digestion, the metals were determined complexometrically.

For the determination of anion exchange capacity the humic acids were precipitated from a 4.5% aqueous solution of sodium humate (pH = 8) with an aqueous solution of 0.1 N hydrogen iodide. The precipitate was allowed to stand for one hour and was separated by centrifugation and its washing was repeated three times. The precipitate was suspended in 10 cm^3 distilled water. Then 3% hydrogen peroxide (2.5 cm^3), two drops of a 50% aqueous solution of phosphoric acid and 1% ammonium molibdate were added to the suspension and

shaken vigorously with 2 cm^3 chloroform. Even in an aqueous solution of starch, no iodine formation could be observed.

For redox potential and redox capacity measurements, 0.05 M hydroquinone (25 cm^3) or 0.1 g humic acid (50 cm^3) were dissolved in distilled water (pH = 3), then titrated with 0.1 N potassium bichromate (in 1 N phosphoric acid) by means of a Radelkis OP-C-7112-D redox electrode.

An excess amount of 0.1 N aqueous potassium bichromate was added to a 0.1 g humic acid acetate buffer (pH = 3) and allowed to stand overnight. The precipitate was separated by centrifugation, decanted and washed. After reduction, the residual excess amount of bichromate detected in the solution mixture was determined iodometrically.

Determination of quinone by stannometry. 0.1 g fine ground humic acid was added to a solution of 0.2 N (7 cm^3) tin(II) chloride (2.26 g $SnCl_2 \cdot 2H_2O$ + 30 cm^3 cc HCl, freshly prepared and made up to 100 cm^3 in a volumetric flask) in a thick glass ampule (10 cm^3). The ampules were sealed, placed in a thermostat and kept at 120°C for hours. A blank sample was simultaneously prepared. After being cooled, the ampules were opened, the product was filtered on paper, washed quantitatively with approx. 40—50 cm^3 water and put into a titration flask. If a negative reaction was obtained for Cl^- ion, after being washed the filtrate was titrated with 0.1 N iodine solution in the presence of a starch indicator.

Acid-alkalimetric titration curves gave high total acid number values for humic acids, which is in agreement with the origin of the humic acids (lowland peat) and the purity of the samples (metal free). Proton-binding ability characterized by the acid number is determined by the mili-equivalent of alkali hydroxide/1 g required for neutralization. The results obtained with barium or calcium hydroxide as well as barium or calcium acetate are not reliable since they do not lead to unambiguous end-products: e.g. in addition to $U^-Ca^{2+}U^-$, U^-- $Ca^{2+}AC^-$ and/or $U^-Ca^{2+}OH^-$ are also formed (LAWSON—BAILEY 1965).

Cation exchange capacity values obtained at different pH values (ranging from pH = 7 to the final pH values given in Table 1) are depicted in Table 1. With the exception of alkali metal ions, the ion exchange capacity values for bi- and multivalent metal ions never reach the total acidity values. Capacity values depend on the nature of the cation and the pH value, as well as on the proton activity of the anion

$$(ClO_4^- < NO_3^- < Cl^- < SO_4^{2-} < HPO_4^{2-} < CH_3COO^- < OH^-).$$

For the elimination of pH- and anion-dependence, the cation exchange capacity was determined in the following set of experiments at constant pH values and high acetate anion concentrations, using an excess amount of sodium acetate-acetic acid buffer (Table 2). As is evident from the data of Table 2, although the ion exchange capacity increases with the increase of the cation charge, the total acid number cannot be reached even in the case of quadrivalent thorium ions. Only trace amounts of chromium(III) aquoions are bound to the humic acids (see Table 1 and our EPR measurements (LAKATOS *et al.* 1978)). A detailed kinetic interpretation of the reaction and the inertness of the chromium(III) hexaaquoion have been dealt with in a previous paper (LAKATOS *et al.* 1977). The high capacity values for chromium(III) ion (Table 2) could be obtained as follows: chromium(III) ion was prepared in an acidic medium from bichromate ion by hydrazine reduction and the "in statu nascendi" formed chromium(III) ion, which was coordinately not totally saturated, was bound to the humic acids according to the charge number (Table 2). A similarly large number of aluminium(III) ions were incorporated into the humic substances. As could be expected, bivalent ions were incorporated in the following order: first the highly complexing 3d-transition metal ions, such as copper(II) and nickel(II) ions, then the other 3d-ions, and finally, alkali earth metal ions. Considering both theoretical aspects and soil research problems, it should be noted that the capacity values for magnesium ions are always higher than those obtained for calcium ions. This may be ascribed

Table 1

Cation exchange capacity values of I- and hydrolyzed humic acids

Metal ion	Types of humic acids	Anion	Final pH value (Initial pH = 7)	Capacity (mequiv/g)
H^+	I-humic acid	Cl^-	2.7	6.0
	hydr. humic acid	Cl^-	2.7	5.0
Cu^{2+}	I-humic acid	ClO_4^-	4.0	4.6
	hydr. humic acid	ClO_4^-	4.0	4.0
Cr^{3+}	I-humic acid	Cl^-	3.5	0
	hydr. humic acid	Cl^-	3.5	0
Fe^{2+}	I-humic acid	SO_4^{2-}	3.2	4.23
	hydr. humic acid	SO_4^{2-}	3.2	4.2
VO^{2+}	I-humic acid	SO_4^{2-}	3.55	3.4
	hydr. humic acid	SO_4^{2-}	3.55	3.3
Mn^{2+}	I-humic acid	ClO_4^-	5.15	3.42
Zn^{2+}	I-humic acid	ClO_4^-	5.3	2.4
		Ac^-		2.95
Co^{2+}	I-humic acid	ClO_4^-	5.75	2.31
Ca^{2+}	I-humic acid	ClO_4^-	4.35	2.4

Table 2

Cation exchange capacity values of different humic substances

Cation	Anion	pH	Ion exchange capacity, mequ/g					
			Fulvic acid	Hymato melanic acid	Brown humic acids			
					I	hydr.	II	III
H^+	Cl^-		8.0	9.0	6.0	5.0	7.5	6.5
Th^{4+}	NO_3^-	3.5	7.25	7.3	4.2	3.8	6.5	5.9
Cr^{3+}	SO_4^{2-}	3.0	7.5	8.9	5.0	4.9	5.2	6.4
Al^{3+}	Cl^-	4.5	5.2	7.9	4.9	3.8	4.3	3.9
Cu^{2+}	ClO_4^-	4	5.7	4.8	4.7	4.2	4.3	3.4
Ni^{2+}	Cl^-	6	5.8	5.2	4.8	3.4	3.2	4.1
Fe^{2+}	SO_4^{2-}	3	5.1	6.3	4.3	4.2	4.2	4.0
Mn^{2+}	Ac^-	6	4.3	5.6	4.0	3.9	3.5	2.3
Zn^{2+}	Ac^-	6.5	4.1	5.5	3.0	2.9	3.9	3.1
VO^{2+}	SO_4^{2-}	3	3.4	5.1	3.4	3.3	2.3	1.4
Co^{2+}	ClO_4^-	6	3.7	5.1	2.4	2.3	3.8	2.3
Mg^{2+}	Ac^-	6	4.9	6.5	4.0	3.4	4.6	2.9
Ca^{2+}	Ac^-	7	4.4	5.2	2.5	2.3	3.5	2.1

to both colloid chemical and structural reasons. Within the limits of our experiments (SIPOS *et al.* 1977), the molecular weight of humic acids linearly increases with the increase of calcium ion concentration. Owing to the considerable aggregation before precipitation, access to the functional groups, and thus further saturation with calcium ions, is difficult. Similar phenomena have been observed by KATCHALSKY (1964) and KATCHALSKY *et al.* (1961), who pointed out that although calcium ions precipitate biopolymer polyelectrolytes (e.g. polyuronic acids: pectic acids, alginic acids with acid number 5.67 mequ/g), no more than 80% of the functional groups can be saturated. With the increase of ionic strength, the metal humates and/or metal polyuronate aggregates are removed from the solution in the form of precipitates before all the functional groups of these biopolymers reach the first coordination sphere of bi- or multivalent metal ions. Since the final bonding of humic acid ligands generally occurs in the first coordination sphere of the metal ions (LAKATOS *et al.* 1978), it may be expected that in the competition between magnesium ions and side-ions (in this case the sodium ions of the acetate buffer system), the magnesium ion, with its smaller radius (0.065 nm), will form a stronger bond than the larger calcium ion (0.099 nm).

The results of the competition between side-ions (e.g. sodium and hydrogen ions) in the case of strongly complexing copper(II) and zinc(II) ions at constant pH values (3, 4, 5 and 6) with an excess of acetate buffer are presented in Table 3. As can be seen, with increasing pH values, hydrogen ion concentration decreases and the sodium ion content considerably increases. The amount of side-ions, e.g. sodium ions, may increase to such an extent that the capacity values of copper(II) ions decrease with the pH value, while those of zinc(II) ions reach their maximum at pH = 4 and then also decrease gradually. Pure metal (side-ion) free humates can be obtained only by the method of ion exchange resins (DESAI *et al.* 1970) described in our first paper (LAKATOS *et al.* 1977).

Table 3

Ion exchange capacity values of II-humate for copper and zinc as a function of pH and sodium ion concentration

Metal-humate	pH	Zn^{2+} and/or Cu^{2+} ion	Na^{+} and H^{+} ion (mequ/g)		Total capacity values
Zn—Hu	3	1.677	0.37	5.5	7.547
Zn—Hu	4	1.912	1.77	3.82	7.502
Zn—Hu	5	1.331	5.33	0.8	7.461
Zn—Hu	6	1.01	5.82	0.7	7.53
Cu—Hu	3	2.289	0.52	4.7	7.509
Cu—Hu	4	2.188	2.77	2.6	7.558
Cu—Hu	5	1.248	4.38	1.9	7.528
Cu—Hu	6	1.124	6.15	0.0	7.274

The capacity values of simultaneously present metal ions (e.g. copper(II) and iron(II) ions) are given in Table 4. As is evident, the capacity values are determined by both the concentration ratios and the relative affinities of the individual ions.

Table 4

Simultaneous determination of III-humic acid capacity values for iron(II) and copper(II) ions

Fe/Cu ratio	Capacity, mequiv/g (from solution)			Metal content, % (from precipitate)		
	Total	Fe^{2+}	Cu^{2+}	Total	Fe	Cu
1 : 1	3.19	0.75	2.44	7.85	1.65	6.2
3 : 1	2.79	1.45	1.34	5.63	2.23	3.4
1 : 3	2.9	0.54	2.36	7.2	1.2	6.0

Our attempt to determine the anion exchange capacity by means of an aqueous solution of 0.1 N hydrogen iodide gave negative results. In accordance with this, Szalay and Szilágyi failed to adsorb iodide ion from the aqueous solution of ^{131}I-labelled potassium iodide onto their "peat product", obtained from Kecel peat by purification with benzene-alcohol and hydrochloric acid. In our previous paper (LAKATOS—MEISEL 1978) reference was made to the ^{35}Cl NQR measurements of LINDQUIST—LINDMAN (1969), according to which there is no chemical bond between chloride ions and humic acid molecules.

Redox potential and capacity determinations. Analytical methods (BOROMISSZA—VARGA 1972) developed for the determination of the quinone content of humic acids (e.g. oxidation of tin (II) into tin (IV)), chemical evidence, e.g. colourless vanadate-ion reduction to blue vanadyl (II) (SZALAY—SZILÁGYI 1967, SZILÁGYI 1970), the measurement of rH values as a function of pH value (FLAIG *et al.* 1968), EPR investigations (LAKATOS *et al.* 1978) and Mössbauer studies (LAKATOS *et al.* 1977) unambiguously point to the semi-quinone character of the Haworth nucleus of humic substances (SCHNITZER—KHAN 1972). To the best of our knowledge, Wissler was the first to attempt the determination of the normal redox potential of humic acids isolated from tropical (African) bog peat (VISSER 1964). This humic acid was reduced with hydrogen in the presence of a colloid palladium catalyst and, after removal of the excess reducing agent, the product was oxidized with potassium ferricyanide in a nitrogen atmosphere in a phosphate buffer (pH = 7). For the different humic acid samples extracted from different depths of peat soils, redox potential values ranging from +0.32 V to +0.38 V were obtained. Szilágyi attempted the determination of the normal redox potential value of the above "peat products" on the basis of the pH dependence of the redox potential values (SZILÁGYI 1971). For the evaluation of the normal redox potential of the heterogeneous peat-water system from the above correlations, the humic acid content of the "peat product" and the concentration ratios of the oxidized and reduced forms of humic acid should have been determined. On the basis of the interaction between different normal redox potential systems and humic acids:

$$Fe^{3+} + e^- \rightleftarrows Fe^{2+} \qquad E_0 = 0.77\ V$$

(SZILÁGYI 1971)

oxidized humic acid + 2e ⇄ reduced humic acid

$$E_0 = ?$$

$$UO_2^{2+} + 4H^+ + 2e \rightleftarrows U^{4+} + 2H_2O$$

$$E_0 = +0.62\ V$$

(SZALAY—SZILÁGYI 1961)

the normal redox potential values of brown humic acids seem to range between +0.6 V and +0.8 V. The normal redox potential values (FLAIG *et al.* 1968) of several substituted benzoquinones as well as Manecke's redox resin (MANECKE 1955) obtained from the polycondensation of hydroquinone, phenol and formaldehyde are also in this range ($E_0 = +0.7$ V) and redox capacity: 4 mequ/g.

The redox capacity values of different humic acids were provided by tin-chloride analytical methods (BOROMISSZA—VARGA 1972). These values are compared with the results obtained from redox electrode titration in Table 5. The redox titration curves of humic acids are similar

Table 5

Redox capacity values of fulvic and humic acids (mequiv/g)

Sample	Redox electrode determination	Analytical methods	
		Chromatometry	Tin chloride
Fulvic acid	1.0	1.0	1.0
II-humic acid	2.5	3.35—3.4	—
III-humic acid	2.0	1.9 —2.2	—
I-humic acid			1.94

to those of the hydroquinone-bichromate system. Due to higher molecular weights, however the curves are somewhat smoother, which also suggests a bi-electron overall change. The value of the redox capacity is quite high, almost as high as that of Manecke's redox resin (MANECKE 1955).

Finally, it should be mentioned that in the case of VO_3^- ion, much lower redox (0.02 mequiv/g) and ion exchange capacity values (0.2 mequiv/g) have been observed for "peat products", which can be mainly attributed to the high contamination of the products (SZALAY—SZILÁGYI 1967).

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Prepared at the Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest.

J. MEISEL, B. LAKATOS, G. MÁDY

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INFLUENCE OF DIFFERENT INTERSTOCKS ON THE SAPLINGS OF SOUR AND SWEET CHERRY

Prunus mahaleb is the most frequent rootstock for sour-cherry and sweet cherry grafts. On *Prunus mahaleb* rootstocks the varieties generally take well and grow vigorously. Several researchers have tried to check the growth using interstems. According to KRAMER (1973), JORDAN (1967) and KRAMER—STÖRTZER (1974) the sour-cherry Pándy is suitable for dwarfing cherry trees when used as an interstock, while BRASE—WAY (1959) and PLOCK (1973) suggest the use of *Prunus fruticosa* for the same purpose. With the latter incompatibility may occasionally occur.

In studying the effect of using interstems it was found that the order of the grafting partners was decisive for the life of the tree (MOSSE 1960).

Grafting produces an artificial symbiosis, the duration of which depends on the partners being able to ensure the necessary nutrients for themselves during the symbiosis with the assistance of the partner. One of the preconditions for lasting symbiosis is, in general, a close relation between the partners (ZAFONOV—VEIDENBERG 1969).

The causes of disturbances in the stock-scion connection are still far from clear. There is no sharp dividing line between incompatibility and compatibility. Incompatibility can best be regarded as a factor which inhibits compatibility, and which arises from a disharmony between the meristemic tissues and biochemical properties of the partners (TUBBS 1973), though ecological factors also play a role in it (GUR 1957).

Table 1

Taking percentage, height and variance of fruit trees grafted in hand using interstocks

Combinations	n	Taking, %	Full height, cm	One year's growth, cm	S^2
1. P2-C500 ∅	160	87	107	107	407
2. P2-P2-C500	57	61	106	96	459
3. P2-C500K-C500	121	43 (58)[x]	44***	34***	125***
4. P2-SL64-C500	160	61	120.5**	110.5*	491
5. P2-C117-C500	160	70.5	98.4*	88.4*	422
6. Germ-C500 ∅	143	69	97	97	437
7. Germ-P2-C500	32	56 (63)[x]	42***	32***	47***
8. Germ-SL64-C500	41	55.5	114*	104	369
9. Germ-C117-C500	64	52	98.6	88.6*	317

[x] Figures in brackets represent the taking percentage in summer.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The present paper also deals with the effects of related plants on the trend of symbiosis. A "C500" seedling population originating from a "C500 K" *Prunus mahaleb* mother tree was used as rootstock, onto which a 10 cm long intermediate stem section and a scion with 2—3 buds on it were grafted in hand in one action (JÁKY 1977). Some of the scion were "Újfehértói fürtös (P2)" sour-cherry, and the rest were "Germersdorfi óriás" cherry. The interstock partners

Fig. 1. Longitudinal section of the upper grafting site of the *Újfehértói fürtös (P2)-C500K—C500* combination a year after grafting

were "C117 Pándy" sour-cherry clone, "Újfehértói fürtös (P2)" sour-cherry clone, "SL64" *Prunus mahaleb* clone, and in one of the combinations "C500 K" *Prunus mahaleb* mother tree clone.

The trees were planted at a nursery spacing of 90×30 cm in calcareous sandy soil and raised using normal nursery methods. The percentage of taking and the height of the

saplings were recorded on 7th September. When comparing the measurements of height, apart from the total height of the sapling only the one-year differences in shoot growth were considered without the length of the interstem (10 cm). The average height of the trees was determined by the method of variance analysis, while the range of the data were compared using the Bartlett test.

Fig. 2. Longitudinal section of the lower grafting site of the *Újfehértói fürtös (P2)-C500K—C500* combination a year after grafting

The growth of the various grafting combinations was noticeably different from that of a single graft in many cases (Table 1). The "SL64" *Prunus mahaleb* interstem combined with scions of both "Újfehértói fürtös" and "Germersdorfi óriás" was conspicuous among the growth averages due to its vigorous growth. If the interstem material was the "C117 Pándy" clone, on the other hand, both scions displayed a significant decrease in growth. In the combination

series for "Újfehértói fürtös"(P2) the sapling with a "C500 K" *Prunus mahaleb* interstock showed very poor growth, for which some kind of incompatibility was obviously responsible. In this case the upper grafting site showed an intensive thickening, while the lower one was very deficient in uniting and in callus formation (Figs 1 and 2). The same was observed in the case of the cherry variety "Germersdorfi" when the sour-cherry "Újfehértói fürtös" was the interstem material. In both cases the upper grafting site was the thicker; here the union was complete, at least as far as callus formation was concerned. Union at the lower end of the interstem section, on the other hand, was deficient, rendering the graft liable to break. The taking percentages of the two combinations which showed incompatibility were relatively good, but in the summer quite a lot of them died.

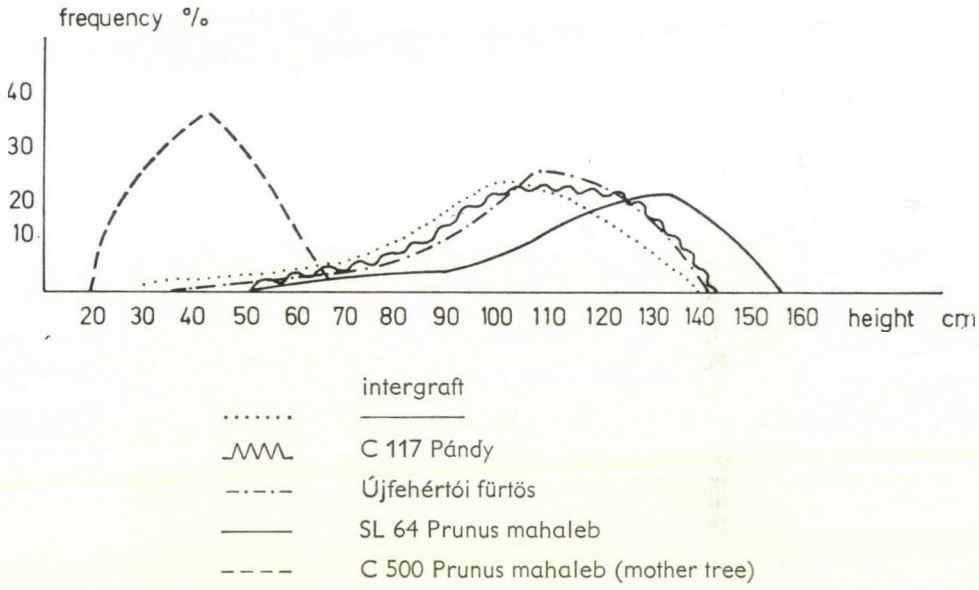

Fig. 3. Effect of intermediate stem sections of different varieties grafted between a "Újfehértói fürtös meggy" scion and a "C500" *Prunus mahaleb* seedling rootstock on the variance in sapling size

When subjected to the Bartlett test the latter two combinations were found to form a more inhomogeneous stand at the $P < 0.001$ level than the other trees, while the other combinations exhibited a convergent range (Figs 3 and 4). When comparing the combinations for variance we find that the character of symbiosis is controlled here by a factor much more powerful than the heterogeneity of the "C500" *Prunus mahaleb* population. The effect of this factor is even stronger than the growth regulation of the stock-scion combination. Although the variation curves deviate to the right or to the left when other rootstocks are used as intermediate stem sections, they do not substantially change in character.

On a *Prunus mahaleb* rootstock sour-cherry and cherry generally unite well. When the varieties used in our investigations are examined separately, contradictions are found. These are the following:

1. "Újfehértói fürtös" sour-cherry and "Germersdorfi óriás" cherry prove to be equally compatible with *Prunus mahaleb* "C500" when grafted to it separately, but the threefold combination shows incompatibility.

2. The closest relation is that between "C500" *Prunus mahaleb* and the "C500K" mother tree, yet the clone of the mother tree causes disorders and disturbs the symbiosis when used as interstem.

3. The use of the clone "C117 Pándy" as interstem material between a "Germersdorfi" scion and a "C500" *Prunus mahaleb* rootstock gives complete union, while "Újfehértói fürtös", which is also of the "Pándy" type, produces symptoms of incompatibility when used as the interstem section.

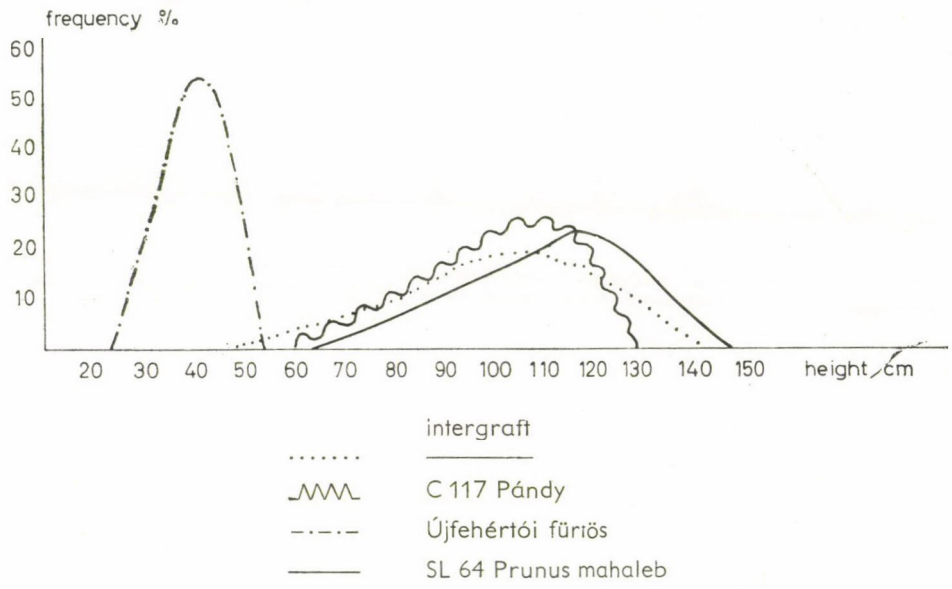

Fig. 4. Effect of intermediate stem sections of different varieties grafted between a "Germersdorfi óriás" cherry scion and a "C500" *Prunus mahaleb* seedling rootstock on the variance in sapling size

4. The taking percentage of the defective grafting combinations is almost normal, while in some combinations which show complete union it is lower (Fig. 5).

5. In both cases the union is deficient at the lower end of the interstem section, where it is complete in the case of a single grafting (e.g. "Germ.-P2-C500" and "P2-C500").

*

Prepared at the Research Station of the Research Institute of Fruit Trees and Ornamental Plants, Cegléd.

A. JÁKY

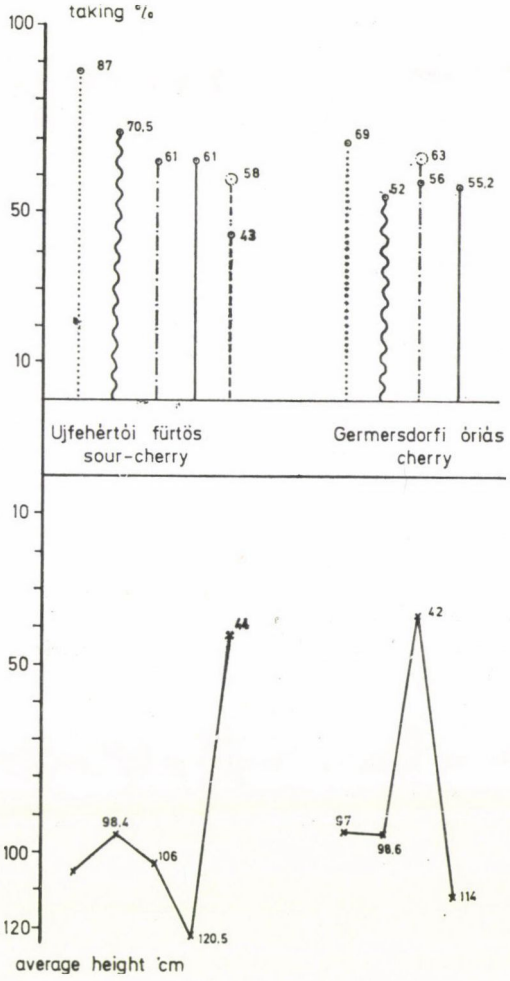

Fig. 5. Taking percentages and average heights of "Újfehértói fürtös" sour-cherry and "Germersdorfi óriás" with different interstems on a "C500" *Prunus mahaleb* rootstock

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ANALYSIS OF VOLATILE OILS IN SALVIA SCLAREA L. FOR THE PURPOSE OF QUALIFICATION. I. THIN LAYER CHROMATOGRAPHY STUDIES

Studies have been carried out on representatives of the *Salvia* genus since 1971, with special regard to *Salvia sclarea* L. The studies included investigations into volatile oil formation in the developing plant (THEN 1972a, THEN—VERZÁR-PETRI 1969, VERZÁR-PETRI—THEN 1975, THEN 1972b), and analyses by thin layer and gas chromatography of volatile oils in the fully developed plant, in an endeavour to find an explanation for the differences between the species (VERZÁR-PETRI—THEN 1974a). In the course of histochemical analyses differences in composition were demonstrated between various types of secretory organs and localization sites (VERZÁR-PETRI—THEN 1974b). In Hungary the volatile oil of *Salvia sclarea* is widely used in the cosmetics industry. The volatile oil obtained from *Salvia sclarea* by steam distillation is yellow; it is highly valuable, as is the ambergris preparation (sclareol) obtained by an extraction technique, which is now available at a very high price. Hungary plays an important role in sage growing. A detailed analysis of the volatile oils used in our country is given in the description of the standard physical and physico-chemical methods (ANONYMOUS), though this gives no information about the concrete qualitative differences between the components (BÉLAFI—RÉTHY *et al.* 1978). Our aim was thus to elaborate, on the basis of our experience and present work, procedures which could also be used for standardization. Further, it was decided that sage oils obtained from various growing sites should be examined as to any difference in composition, in order to call attention to possible taxonomic questions. Differences of this kind may involve peculiarities important from the point of view of manufacturing technology, since they may affect the quality of the product.

Finally, it was hoped to determine the influence exercised by the volatile oil content in the different parts of the flower (bracts, petals, carpels) on the composition, and possibly quality of the oil, and with the information thus obtained to work out a procedure to replace the extremely expensive absolute production technology currently used in Hungary.

The oils examined were obtained from *Salvia sclarea* L. Labiatae grown and steam distilled at Darányipuszta, Herceghalom and Biatorbágy. Fractional separation was also carried out by glycol distillation of the material obtained from Darányipuszta. All the samples originated from plant material harvested in 1975. The flowering shoots used for further detailed analyses were collected on 10th June 1976 at Herceghalom.

The methods of analysis used were: thin layer chromatography according to STAHL (1962), gas chromatography (VERZÁR-PETRI—THEN 1974a), fractional steam distillation and petroleum ether extraction. Working on an aqueous Kieselgel G (Merck) carrier, 0.25 m/m in thickness, without activation, the analyses were based on the application of various developing mixtures and on the use of conc. H_2SO_4 — vanillin developing agent, which all proved to be suitable on the basis of terpene examinations published in the literature (SCHRANTZ 1957, SPRECHER 1963, LOOMIS 1967, VON SCHANTZ—HUHTIKANGAS 1971).

1. *Methodological observations*

a) The physico-chemical properties of *Salvia sclarea* oils obtained from different places are summarized in Table 1. No essential differences have been found. b) With respect to the five one-dimensional and one two-dimensional methods of thin layer chromatography employed, the following experiences have been gained:

Hexan developer: this was definitely unsuitable for the separation of oil components.

Table 1

Physico-chemical quality parameters of volatile oils in Salvia sclarea

Origin	Colour	Specific weight	Refraction index	Optical activity	Solubility. Volume in 90° alcohol	Density, s	Σ	Ester content, %
Daránypuszta quality I	brown	0.901/20°C	1.470	−8°	0.5	1.2	120.6	42.2
Darány-puszta quality II	brownish yellow	0.899/20°C	1.4629	−11.1°	1.0	0.8	130.2	45.58
Daránypuszta commercial	yellowish brown	0.8998/20°C	1.4711	−5.7°	0.5	0.11	154.01	53.9
Hercegh-halom 1	greenish	0.8971/20°C	1.4662	−11.6°	0.5	0.57	180.95	63.33
Hercegh-halom 2	yellowish brown	0.896/20°C	1.473	−11.2°	0.5	1.1	135.48	47.41
Páty 1	yellowish brown	0.9031/15°C	1.46	−10.6°	0.5	0.47	186.77	65.35
Páty 2								
Biatorbágy 2	dark yellowish brown	0.898/20°C	1.4870	0	0.51	0.86	119.3	41.7
commercial control (E)	yellow	0.899/19°C	1.4690	−25.6°	0.5	0.38	139.64	48.87

Chloroform developer: Stahl recommends this for the separation of terpene esters and terpene alcohols; the esters show higher R_f values. Each of the Herceghalom samples, the Biatorbágy sample and that accepted by the original standard showed a difference in a single yellow component compared to the Páty and Darányпuszta samples; in other components no difference was observed. The differing component was not examined further.

Benzol developer: in esters and terpene alcohols the order of separation was opposite to that given above. There were differences between the Páty, Biatorbágy and Darányпuszta samples: some new components appeared in them.

Benzol : ethyl acetate (95 : 5 or 90 : 10): Stahl recommends this as a general developer for volatile oils. In our experience it is suitable for the uniform expansion of the spectra in the analyses (Figs 1, 3 and 4). Our observations agree with Stahl's opinion.

Benzol : dioxan : glacial acetic acid (90 : 25 : 4): According to Stahl this developer is specific for acids. We found that the alcoholic type components were also well defined; the lower range is clearly separated.

The results obtained with the different developers are summarized in Table 2.

Table 2

Components of sage oil demonstrated by thin layer chromatography

Components identified by test material	Developers							
	Hexan		$HOCl_3$		Benzol : ethyl acetate 95 : 5		Benzol : dioxane : glacial acetic acid 90 : 25 : 4	
	R_f	colour	R_f	colour	R_f	colour	R_f	colour
Linalool	0.18	brown	0.43	green red	0.61 0.53	green	0.74 0.63	green
Linalil acetate	0.36	brown	0.68	reddish lilac	0.81	green	0.86 0.82	green
Geraniol	0.13	brown	0.36	bluish green	0.50	green	0.61	green
				greenish blue	0.4	yellow yellow	0.72 0.4	yellow yellow
Nerol	0.15	brown	0.36	green	0.41	green	0.56	green
Borneol	—	—		yellow	0.58	yellow	0.69	yellow
-pinene	—	brown	0.18	bluish green	0.73	yellow	0.70	yellow
-pinene	—	brown	0.87	green	0.81	green	0.89	green
Terpineol	—	—	0.31	greyish green	0.52 0.43	dirty violet	—	—
Terpinil acetate	0.36	—	0.68	green lilac	0.75 0.42	dirty violet	—	—

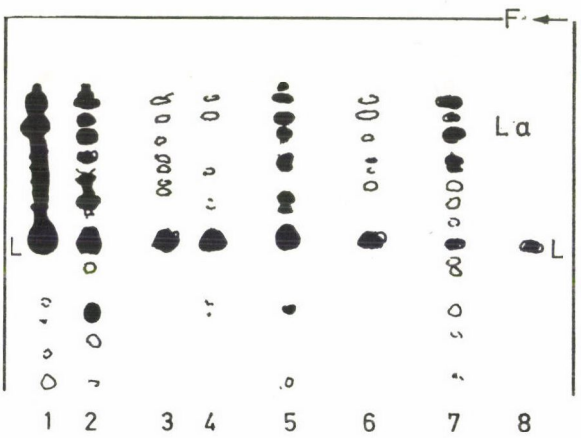

Fig. 1. One-dimensional thin layer chromatogram of *Salvia sclarea*. Benzol : dioxane : glacial acetic acid = 90 : 25 : 4. Developer: vanillin in alcoholated conc. sulphuric acid; 1 = bud extr., 2 = flower extr., 3 = petal extr., 4 = calix extr., 5 = hypsophyll extr., 6 = floral axis extr., 7 = leaf extr., 8 = leaf epidermis extr.

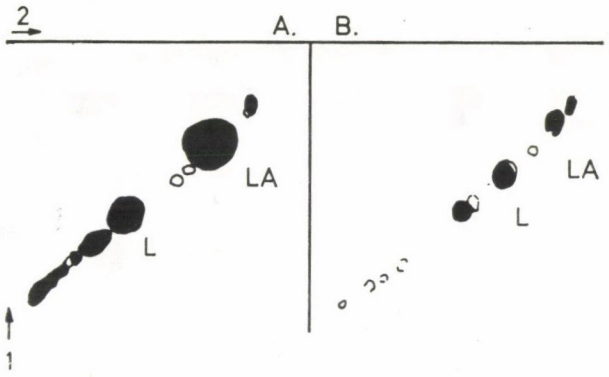

Fig. 2. Two-dimensional thin layer chromatogram of sage oil; 1 = linalool, 2 = linalil acetate. Directions of development indicated by arrows

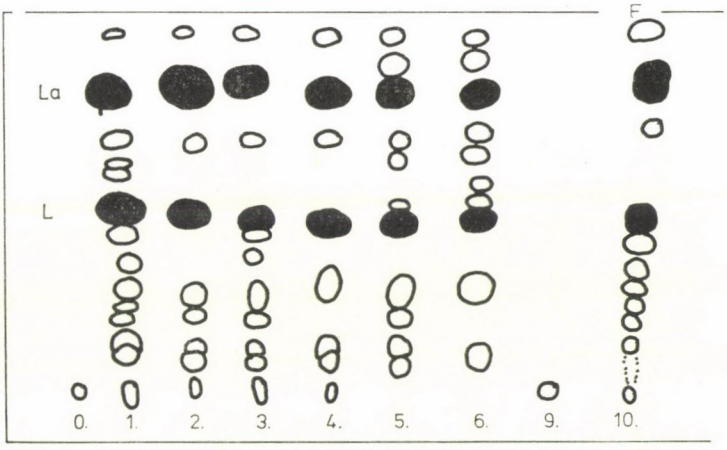

Fig. 3. Thin layer chromatograms of various *Salvia sclarea* oils. Benzol : ethyl acetate (90 : 10); 0 = flower, 1 = distillate (1), 2 = distillate (2), 3 = distillate (3), 4 = solvent distillation, 5 = solvent distillation, 6 = solvent distillation, 9 = flower, 10 = *Salvia* volatile oil diluted to 10%, 11 = flower, L = linalool, La = linalil acetate

The two-dimensional chromatographic examinations were performed with benzol : ethyl acetate developer run in two directions; here the results obtained previously were confirmed, but further components did not appear (Fig. 2). The evaluation thus achieved was not sufficiently exact to make the more precise gas chromatographic examination superfluous. The results of gas chromatographic examinations will be published in a later paper.

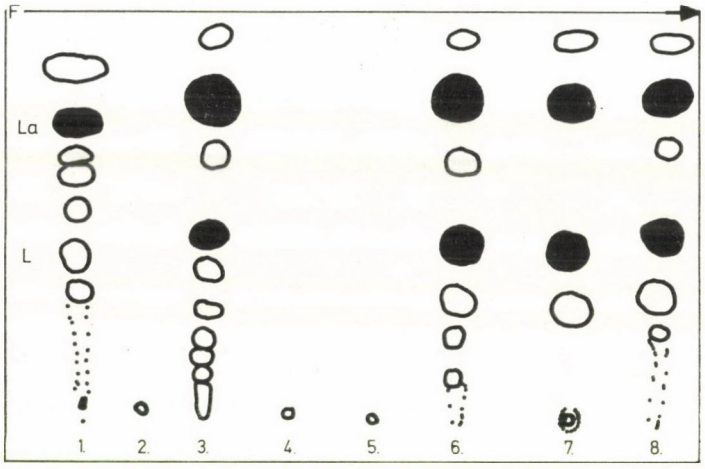

Fig. 4. Thin layer chromatograms of *Salvia sclarea* oils. Benzol : ethyl acetate (90 : 10); 1 = Sclarambrol, 2 = Salviron, 3 = Res. Souge scl., 4 = Res. abs. Souge, 5 = Abs. Souge, 6 = *Salvia sclarea* volatile oil dist. (distillate 1), 7 = *Salvia sclarea* dist. (distillate 2), 8 = *Salvia sclarea* dist. (distillate 3)

2. *Examination of volatile oil fractions*

In the second part of this work the various fractions achieved by fractional separation were analysed (Fig. 1). From the first fraction bornil acetate, geranil acetate and neril acetate components were identified. In the second fraction borneol appeared and in the third nerol was found (Table 3). In the residue fraction nerol and borneol were found, together with substances which have higher boiling points.

3. *Identification of perfume compositions*

Our third subject was to identify perfume compositions prepared from *Salvia sclarea* oil imported from abroad. This proved to be very difficult, as the components differed in part from those of the *Salvia sclarea* plant. Only the linalil acetate spot could be reliably identified, while the presence of linalool and geraniol was only assumed. Thin layer chromatography was not found to be suitable for the identification of the other components. The analyses must be made more exact with GC and with spectroscopic analysis after isolations.

4. *Results of analysing the flowering shoot*

The calix contains the lowest number of components, which are identical to those in the hypsophyll. There is a larger quantity of sesquiterpene in the leaf. Extraction samples prepared from the green sepal and from the petal also show differences in the range of sesquiterpene and terpenic acid. These results confirm the histological analyses performed so far, in which the distribution of glandular hairs showed a zonal pattern (Fig. 1).

In the course of our investigations we found only slight differences between the oils of *Salvia sclarea* plants obtained from various growing sites, which shows the homogeneous nature of this plant in Hungary. The differences may be the result of the extraction method. For a quick analysis thin layer chromatography is the best method. As a developer the most

commonly used agent, benzol : ethyl acetate (95 : 5), is still to be recommended; linalool and linalil acetate can be reliably demonstrated using thin layer chromatography, and in our opinion these are sufficient for the qualification. The question of differentiated collecting promises to be interesting, but requires further study.

Our present investigations are of an informative character, but if the results thus obtained are supplemented by further experiences, they should prove useful in control experiments too.

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Prepared at the Cosmetic and Household Chemistry Enterprise, Budapest; Institute of Medicinal Plants and Drugs of the Semmelweis Medical University, Budapest.

M. THEN, G. VERZÁR-PETRI

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ULTRASONIC DETERMINATION OF BONY MEAT AND LARD CONTENTS OF VALUABLE PARTS OF THE BODY IN LIVE HOGS

The qualification system used in slaughter-houses gives preference to porkers showing a high proportion of lean meat rather than to fat porkers giving a large quantity of lard. This fact encourages the farms to produce slaughter animals supplying large volumes of meat and only a small amount of lard. Pig farms must therefore be aware of the meat-producing capacity of their stocks and have an exact knowledge of the good breeding porkers, so as to be able to continuously improve the quality in the interests of both the farm itself and the national economy. As the h^2 value of the slaughter characteristics is relatively high ($h^2 = = 0.5—0.7$), it is possible to achieve considerable improvements in this field in a comparatively short time by means of proper selection. In this work the breeder is greatly helped by any simple, rapid and accurate method suitable for assessing the amount of meat and lard in live hogs. The oldest and most usual method of assessing the slaughter value of live hogs is to judge by their external appearance. It has been found, however, that in the new type of porkers — unlike the previous lard-producing pigs — this method gives rather uncertain indications of the slaughter qualities.

CLAUSEN (1959) called attention in 1953 to the fact that "in fully developed breeds judging the slaughter quality of pigs by carriage has only a secondary role". BRATZLER—MAGERUM (1953), OTTO (1958), WENIGER (1961) and LÖRTSCHER—GERWIG (1959) were of the same opinion, stating that a subjective judgement was not sufficient to establish the quantitative and qualitative composition of the carcass.

Various methods ranging from different body measurements (HETZER 1950, WITT 1967, etc.) to X-ray measurements (HOFMANN—RITTER 1960, HORST 1971, etc.) and isotope examinations (KULWICH *et al.* 1958, etc.) have been employed in an effort to determine the quantity of meat and lard; at present, however, the method of ultrasonic measurement seems to be the most suitable way of solving this problem.

For the last twenty to twenty-five years many authors have dealt with this method, examining it from different aspects. For example, LAUPRECHT *et al.* (1960), RITTLER *et al.* (1964), VANGELOV *et al.* (1971), ANTAL—PASCHKE (1973), PEDRON *et al.* (1974) and others presented data on the precision of the measurements and the factors which influence it. It can be established from these data that with an ultrasonic apparatus the thickness of the fatty tissue in live higs can generally be determined with an 85—95% accuracy, and the thickness of the muscular tissue with an 80—90% accuracy. The accuracy of measurement is primarily influenced by the sites of measurement, the thickness of the measured tissue and the posture of the animal. The correlations between the thickness of lard and muscles, as well as between the amounts of meat and lard, were studied by LAUPRECHT *et al.* (1965), BLENDL—SIDOR (1970), ADAMS *et al.* (1972), FALKENBERG—PFEIFFER (1975) and others, who established correlation values of 0.4—0.7.

We have no exact knowledge of the reliability of the data obtained by ultrasonic measurements on the quantity of bony meat and lard for the different types of breed produced in Hungary, despite the fact that this is one of the most important components of the slaughter value. Apart from this, there are some details of the ultrasonic method which require further investigation. Therefore the aim of our present work was to examine: 1. the reliability with which the amounts of meat and lard in Hungarian white porkers can be assessed, 2. the possibility of improving the accuracy of measurement, 3. how conclusions can be drawn from ultrasonic measurements on the amounts of bony meat and lard, 4. the correlations between the ultrasonic measurements made after 24 hours of cold storage on carcasses cut in half and the amounts of bony meat and lard found in the valuable parts of the body, and 5. which sites of measurement show the closest correlation with the amounts of bony meat and lard.

The examinations were carried out between 1973 and 1975 at the Central Performance Testing Stations of the National Inspectorate for Animal Husbandry in Kecskemét-Miklóstelep and Atkár. During that period measurements were made on 69 Hungarian white porkers (39 barrows and 30 sows) each weighing 90 kg. The animals were transferred from various farms to the stations for the purpose of progeny testing when they weighed 26—28 kg. The progeny tests were performed on 6 male and 6 female piglets per boar between the weight limits of 30 and 90 kg.

The pigs were fattened as prescribed by the NIAH standard then in effect. Pigs which had reached a weight of 90 kg (± 0.5 kg) were slaughtered in the slaughter-house attached to the station and, after 24 hours of cold storage, cut up according to the quality requirements; this was done partly as required by the bacon quality standard, and partly taking into consideration certain additional cuts necessary for our examinations.

In the course of the experiment measurements were made on all the pigs in four ways: *a)* on the live animal with ultrasound and a tape-measure, *b)* with a ruler after slaughtering, before and after cutting, *c)* with ultrasound on half-carcasses after 24 hours of cold storage, and *d)* with a ruler on half-carcasses after 24 hours of cold storage.

a) Ultrasonic and tape-measure measurements were carried out on live hogs in the slaughter-house immediately before slaughtering. While they were measured the animals stood in a stall of our own design where the desired posture was ensured by fixing the head and the trunk. Nine measurements were taken of the thickness of lard, five of the thickness of the muscular tissue and two of the length of the animal, all with an accuracy of ± 1 mm, partly above the spinal chord, and partly on the left side of the animal. The points of measurement are shown in Fig. 1.

The sites of measurement were marked on the body by scratching the skin.

b) Measurements taken at points S_1 and S_2 were checked immediately after slaughtering and scalding, but before cutting, on pigs lying on their left sides — being thus very similar to standing animals as regards the position of the bones. For technical reasons measurements obtained at points H_1, H_2, H_3, B_1, S_3 and L_1 were checked after cutting on the hanging half-carcasses (the thickness of the dorsal fat, for example, can only be measured with a ruler on carcasses cut in half).

c) After 24 hours of cold storage the thickness of the fatty and muscular tissues was again measured with an ultrasonic thickness gauge at points S_1, S_2, S_3 and I_{r1} of the left half-carcass.

d) The ultrasonic measurements were also checked with a ruler.

After the ultrasonic and ruler measurements had been made and the hogs cut into pieces the weights of bony meat and hide + lard found in the individual valuable parts of the body were determined with an accuracy of ± 10 g. Apart from these data the age of the hogs when slaughtered was also recorded.

In addition various averages, ratios and percentages were calculated from the basic data obtained in the course of measurement, by combining the data for one or more points of measurement. The basic and calculated data were divided into two large groups.

A) The data obtained with the ultrasonic thickness gauge and the ruler, as well as those calculated from them, formed the independent variables (measurement indices). These are contained in Table 1.

B) The basic data on the quantities of bony meat and hide + lard found in the different parts of the body, as well as those calculated from them, formed the dependent variables. These are contained in Table 2.

After a preliminary processing of the measurement results there was a total of 16,407 data, which could only be processed using a computer. Processing was therefore carried out at the NIAH Computer Centre using an R-100 type computer and the following values were determined:

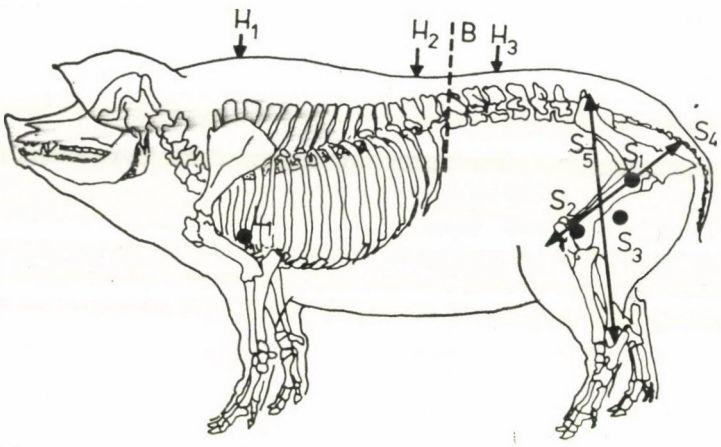

Fig. 1. Points of measurement on live hogs

Point of measurement	Site of measurement	Tissue measured
H_1	above the spine, on the withers where the layer of fat is the thickest	thickness of hide + fatty tissue
H_2	above the spine, on the back, where the layer of fat is the thinnest	thickness of hide + fatty tissue
H_3	above the spine, where the muscles of the loins start	thickness of hide + fatty tissue
B	at the last rib where the side of bacon is the thinnest	thickness of hide + fatty tissue thickness of muscular tissue (chop diameter)
S_1	at the joint of the pelvis and femur immediately below the head of the femur, on the femur	thickness of hide + fatty tissue, thickness of muscular tissue (distance from the border of fatty and muscular tissue to the femur)
S_2	above the knee-joint on the femur	thickness of hide + fatty tissue, thickness of muscular tissue (distance from the border of fatty and muscular tissue to the bone)
S_3	at the roundest point of the ham (at a nearly equal distance from points S_1 and S_2)	thickness of hide + fatty tissue, thickness of muscular tissue (distance between the border of fatty and muscular tissue, and the membrane covering the inside of the ham)
S_4	distance between the knee-joint and the tail-base	length measured with a tape
S_5	vertical distance between spine and ham-bone	length measured with a tape
L_1	between the elbow and the acromion immediately behind the humerus	thickness of hide + fatty tissue, thickness of muscular tissue to the shoulder-blade)

Table 1

Data obtained with ultrasonic thickness gauge and ruler, and those calculated from them

Independent variables (parameters)	Code number for independent variables			
	ultrasonic measurement on live hog, A	ruler measurement on slaughtered hog, B	ultrasonic measurement after 24 hours of refrigeration, C	measurement by ruler after 24 hours of refrigeration, D
Thickness of lard on the withers point H_1	A_1	B_1	—	—
Thickness of lard on the back point H_2	A_2	B_2	—	—
Thickness of lard on the loins point H_3	A_3	B_3	—	—
Average thickness of lard on the back $\frac{H_1 + H_2 + H_3}{3}$	A_4	B_4	—	—
Thickness of the side of bacon point B	A_5	B_5	—	—
Chop diameter point B	A_6	B_6	—	—
Thickness ratio of muscle to lard at point B	A_7	B_7	—	—
Average thickness of dorsal and side bacon $\frac{H_1 + H_2 + H_3 + 2B}{5}$	A_8	B_8	—	—
Thickness of fat on ham point S_1	A_9	B_9	C_9	D_9
Thickness of muscle on ham point S_1	A_{10}	B_{10}	C_{10}	D_{10}
Thickness ratio of muscle to fat at point S_1	A_{11}	B_{11}	C_{11}	D_{11}
Thickness of fat on ham point S_2	A_{12}	B_{12}	C_{12}	D_{12}
Thickness of muscle on ham point S_2	A_{13}	B_{13}	C_{13}	D_{13}
Thickness ratio of muscle to fat at point S_2	A_{14}	B_{14}	C_{14}	D_{14}
Thickness of fat on ham point S_3	A_{15}	B_{15}	C_{15}	D_{15}
Thickness of muscle on ham point S_3	A_{16}	B_{16}	C_{16}	D_{16}
Thickness ratio of muscle to fat as point S_3	A_{17}	B_{17}	C_{17}	D_{17}
Average thickness of fat at points S_1 and S_2 $\frac{S_1 + S_2}{2}$	A_{18}	B_{18}	C_{18}	D_{18}
Average thickness of muscle at points S_1 and S_2 $\frac{S_1 + S_2}{2}$	A_{19}	B_{19}	C_{19}	D_{19}
Average thickness of fat at points S_1, S_2 and S_3 $\frac{S_1 + S_2 + S_3}{3}$	A_{20}	B_{20}	C_{20}	D_{20}
Average thickness of muscle at points S_1, S_2 and S_3 $\frac{S_1 + S_2 + S_3}{3}$	A_{21}	B_{21}	C_{21}	D_{21}
Ratio of average muscle to fat thickness at points S_1, S_2 and S_3	A_{22}	B_{22}	C_{22}	D_{22}
Distance of knee from tail-base point S_4	A_{23}	—	—	—
Distance of ham-bone from spine point S_5	A_{24}	—	—	—
Ratio of values obtained at points S_5 and S_4	A_{25}	B_{25}	—	—
Thickness of fat on shoulder point L_1	A_{26}	B_{26}	C_{26}	D_{26}

Table 1 continued

Independent variables (parameters)	Code number for independent variables			
	ultrasonic measurement on live hog, A	ruler measurement on slaughtered hog, B	ultrasonic measurement after 24 hours of refrigeration, C	measurement by ruler after 24 hours of refrigeration, D
Thickness of muscle on shoulder point L_1	A_{27}	B_{27}	C_{27}	D_{27}
Thickness ratio of muscle to fat at point L_1	A_{28}	B_{28}	C_{28}	D_{28}
Average thickness of fat $\frac{H_1+H_2+H_3+2B+S_1+S_2+S_3+L_1}{9}$	A_{29}	B_{29}	—	—
Average thickness of muscle $\frac{B+S_1+S_2+S_3+L_1}{5}$	A_{30}	B_{30}	—	—
Ratio of average muscle to fat thickness	A_{31}	B_{31}	—	—
Ratio of average muscle to fat thickness at points S_1 and S_2	A_{32}	B_{32}	—	—

Table 2

Basic data showing the quantities of bony meat and hide + lard in different parts of the carcass and the data calculated from these

Dependent variables	Code number for dependent variables
Weight of bony meat in the left-side hindleg ham	y_1
Weight of hide + lard in the left-side hindleg ham	y_2
Weight ratio of bony meat to hide + lard in the left-side hindleg ham	y_3
Amount of bony meat in the left-side hindleg ham as a percentage of the total weight of ham	y_4
Total weight of bony meat in the left-side ham, shoulder and chop	y_5
Total weight of lard in the left-side ham, shoulder and chop	y_6
Ratio of total weights of bony meat to lard in the left-side ham, shoulder and chop	y_7
Total amount of bony meat in the left-side ham, shoulder and chop as a percentage of the live weight	y_8
Total amount of hide + lard in the left-side ham, shoulder and chop as a percentage of the live weight	y_9
Total amount of bony meat in the left-side ham, shoulder and chop as a percentage of the slaughter weight	y_{10}
Total amount of hide + lard in the left-side ham, shoulder and chop as a percentage of the slaughter weight	y_{11}
Weight of bony meat in the left shoulder	y_{12}
Weight of hide + lard in the left shoulder	y_{13}
Weight ratio of bony meat to hide + lard in the left shoulder	y_{14}
Weight of bony meat in the left chop	y_{15}
Weight of hide + lard in the left chop	y_{16}
Weight ratio of bony meat to hide + lard in the left chop	y_{17}

a) the mean, the range (*s*) and the variation coefficient (cv%) for each independent ($\overline{A}$, $\overline{B}$, $\overline{C}$, $\overline{D}$) and dependent variable ($\overline{y}$),

b) mm and % differences between the ultrasonic data and the control measurements obtained with a ruler at the different points of measurement,

c) Bravis correlation coefficients (*r*), regression values (*b*) and the relevant linear equations between all dependent and independent variables.

(Since the above data can only be correctly interpreted in the case of linear function relations, the linearity was checked by drawing graphs for some pairs of characteristics as a random sampling test; the expected positive results were obtained.)

On the grounds of basic data supplied by the computer it was possible to determine the confidence limits of the individual correlation coefficients, and of the estimated *y* values obtained by means of various linear equations.

a) The values of the dependent variables indicate that the amounts and proportions of bony meat and lard found in the hogs included in the experiment can be considered satisfactory compared to the mean for the breed (Table 3). It was important to know the range

Table 3

Data on the mean range and variation coefficient values (dependent variables) which refer to the amounts of bony meat and lard found in the valuable parts of half-carcasses

Dependent variables (code number)	Average, $\overline{X}$	Range, s	Variation coefficient, CV %
y_1	7.69 kg	0.74	9.6
y_2	2.35 kg	0.35	14.8
y_3	3.37	0.77	22.8
y_4	73.27%	3.82	5.2
y_5	15.18 kg	1.16	7.6
y_6	5.08 kg	0.76	14.9
y_7	3.10	0.63	20.3
y_8	16.88%	1.28	7.5
y_9	5.66%	0.83	14.6
y_{10}	22.54%	1.40	6.2
y_{11}	7.59%	1.14	15.0
y_{12}	3.53 kg	0.30	8.4
y_{13}	1.12 kg	0.21	18.7
y_{14}	3.27	0.58	17.7
y_{15}	3.98	0.35	8.7
y_{16}	1.63	0.40	24.5
y_{17}	2.59	0.69	26.6

and variation coefficient values of the individual characters because any unusually large deviation, in either direction, from the range values characteristic of the breed may result in incorrect correlation and regression coefficients. In the stock examined such deviations did not occur; the computed correlation and regression coefficients are thus reliable.

b) The differences between the ultrasonic results and the control measurements obtained with a ruler were, with a few exceptions, less than 1 mm. This means that ultrasonic measurements can certainly be regarded as reliable from the point of view of accuracy. It is interesting that, by contrast to the literature, in our experiment the thickness of lard on the withers could be measured more precisely than on the back or at the loins, though the differences here were also insignificant. This can probably be explained by the fact that in the stall mentioned above the trunk and head of the animal could be fixed, thus greatly promoting the accuracy of measurement. The greatest differences were obtained when measuring the thickness of the lard and muscle at the shoulder-blades.

c) On the basis of the correlation values between the individual dependent and independent variables indices were chosen as the dependent variable (y) so that the different y-values gave the highest positive and negative correlation values.

Dependent variables from y_1 to y_4 express the slaughter value of the hindleg ham in different ways. On slaughtered animals the closest correlation ($r = 0.78$) was shown by measurements taken with a ruler, using the y dependent variables (B). Calculations from measurements made on live hogs by means of an ultrasonic thickness gauge (A), and after 24 hours of cold storage with an ultrasonic gauge (C) and a ruler (D) produced correlation values some 0.10—0.15 lower than the values obtained with method B. It should be noted that the index determined on live hogs with an ultrasonic gauge

$$\left(A_{29} = \frac{\text{thickness of lard measured by ultrasound on withers}}{9} + \right.$$
$$\left. + \frac{\text{back} + \text{loins} + 2 \times \text{sides} + \text{ham}_1 + \text{ham}_2 + \text{ham}_3 + \text{shoulder blades of live hogs})}{9}\right)$$

and the weight of bony meat in the ham (y_1) show a correlation of $r = -0.66$, which is significant at the $P < 0.001$ level. The confidence limits of the r value at the $P < 0.05$ level are $r_{h_1} = 0.51$ and $r_{h_2} = 0.78$.

In estimating the y value belonging to a definite x value the limits of error at the $P < < 0.05$ level are $y + 1.11$ and $y - 1.11$.

This method of measurement is suitable for obtaining information on the amount of bony meat found in the hams of live hogs. The regression equation of y_1 with the A_{29} measuring index is $y_1 = 11.44 - A_{29} \times 0.1936$.

The dependent variables y_5 to y_{11} characterize the joint slaughter value of the ham, shoulder blades and chops in different ways. Here, as for the ham, the highest correlation values were obtained in all cases with method B (measurements taken with a ruler after slaughtering) ($r = 0.83$). The r values obtained with methods A (ultrasonic measurement on live hogs) and D (measurement with a ruler after 24 hours of cold storage) were found to be lower by 0.10—0.15, while those determined with method C (by ultrasound after 24 hours of cold storage) were 0.20—0.30 lower than the r values obtained with method B.

According to the calculations the following values can be determined with a high degree of accuracy by means of ultrasonic measurements on live hogs:

a) The total weight of hide + lard in the left-side ham, shoulder and chop. (A_2 = thickness of dorsal fat measured with ultrasound in live hogs; $r = 0.78$; $r_{h_1} = 0.64$; $r_{h_2} = 0.85$ $y_6 = 0.36 + A_2 \times 0.2351$).

Limits of error in estimating the y value $= y + 0.95$; $y - 0.95$.

b) The ratio of bony meat to hide + lard in the left-side ham, shoulder and chop. (A_{29}; $r = 0.77$; $y_7 = 6.76 - A_{29} \times 0.1941$) $r_{h_1} = 0.64$; $r_{h_2} = 0.85$.

Limits of error in estimating the y value $= y + 0.78$; $y - 0.78$.

c) Total amount of hide + lard in the left-side ham, shoulder and chop as a percentage of the live weight. (A_{29}; $r = 0.77$; $y_9 = 0.82 + A_{29} \times 0.2558$) $r_{h_1} = 0.64$; $r_{h_2} = 0.85$.

Limits of error in estimating the y value $= y + 1.03$; $y - 1.03$.

d) Total amount of lard in the left-side ham, shoulder and chop as a percentage of the slaughter weight. (A_{29}; $r = 0.77$; $y_{11} = 0.90 + A_{29} \times 0.3536$)

$r_{h_1} = 0.64$; $r_{h_2} = 0.85$.

Limits of error in estimating the y value $= y + 1.42$; $y - 1.42$.

The dependent variables from y_{12} to y_{14} refer to the slaughter value of the shoulder. The low r values obtained ($r = 0.4$) show that the amount of bony meat found in the shoulder (y_{12}) cannot be reliably assessed by any of the methods. The situation is somewhat more favourable with the weight of hide + lard in the shoulder (y_{13}), as in this case the r value (—0.62) determined for the A_{22} index (A_{22} = ratio of average fat thickness to muscle thickness measured with ultrasound at points ham_1, ham_2, and ham_3 in live hogs) and the $r = -0.63$ value determined for the B_{14} index (B_{14} = ratio of muscle to fat thickness measured with a ruler at point ham_2 in the slaughtered animal) indicate a medium correlation. Compared to the above a remarkably close correlation was found between the weight ratio of bony meat to hide + lard (y_{14}) found in the shoulder and the measurements obtained with the A_{29} index ($r = -0.76$; $r_{h_1} = 0.64$; $r_{h_2} = 0.85$). This correlation value is sufficient for determining the slaughter value of the shoulder in the live animal with the aid of ultrasonic measurements, by using the regression equation $y_{14} = 6.64 - A_9 \times 0.1784$. The limits of error in estimating the y value are $y + 0.75$ and $y - 0.75$.

The dependent variables from y_{15} to y_{17} characterize the slaughter value of chops in different ways. As in the case of the shoulder no reliable conclusions can be drawn on the amount of bony meat in the chop (y_{15}) from data obtained by any of the measuring methods. On the other hand, the amount of hide + lard (y_{16}) and especially the weight ratio of bony meat to hide + lard (y_{17}) can be satisfactorily determined with method B_{29} (B_{29} = thickness of lard measured with ultrasound at the withers + back + loins + 2× sides + ham_1 + ham_2 + + ham_3 + shoulder_1 in live hog : 9) (in the order in which they are listed $r = 0.74$; $r = 0.84$), and with method A_{29}, which is even more important for us (in the order in which they are listed $r = 0.68$; $r_{h_1} = 0.51$; $r_{h_2} = 0.78$; $r = 0.77$; $r_{h_1} = 0.64$; $r_{h_2} = 0.85$).

The regression equations are:

$y_{16} = 0.43 + A_{29} \times 0.1095$.

Limits of error in estimating the y value: $y + 0.36$; $y - 0.36$.

$y_{17} = 6.62 - A_{29} \times 0.2136$.

Limits of error in estimating the y value: $y + 0.86$; $y - 0.86$.

In an analysis where the indices were compared to each other the superiority of index A_{29} as regards the reliability of estimation, indicated by the r values, was imdisputable.

Taking into consideration the correlation values of this index it can be seen (Table 4) that of all the points of measurement examined this parameter is the most suitable for drawing conclusions on the slaughter value of live hogs, or more precisely, on the amounts of bony meat and lard. This statement is also confirmed by the results of the control (B) measurements.

It must not be forgotten, however, that index A_{29} is the average of 8 measurements of lard thickness (3 on the back, 3 on the ham, 1 on the side and 1 on the shoulder), so that ultrasonic measurement takes more time than usual. Therefore, this method of measurement should be used primarily at testing stations, for performance tests on boars, where the correct estimation of the slaughter value of the hogs examined is very important, and where it is desirable to improve the accuracy even at the expense of increasing the time spent on the measurements. Breeding carried out on the farms, on the other hand, requires a much simpler method of ultrasonic measurement, enabling the slaughter value of 80—100 young pigs to determined each day. From this point of view ,the application of index 4, which is placed second in method A, seems to be the most expedient (Table 5). By means of index A_4, with which measurements can be made simply and quickly (average of lard thickness measured

Table 4

Those features characterizing the slaughter value which show the closest correlation with parameter A_{29}

Features characterizing the slaughter value (dependent variables: y)	Closeness of correlation, r value
y_6	+0.77
y_7	−0.77
y_9	+0.77
y_{11}	−0.76
y_{17}	−0.77

Table 5

Those features characteristic of slaughter value which show the closest correlation with parameter A_4

Features characterizing the slaughter value (dependent variables: y)	Closeness of correlation, r value
y_6	+0.72
y_7	−0.72
y_9	+0.71
y_{11}	+0.71
y_{17}	−0.73

on withers, back and loins by ultrasound) the value of $y_6/(y_6 =$ weight of lard in the left-side ham, shoulder and chop; $r = 0.71$) and of y_{17} (y_{17} = weight ratio of bony meat to hide + lard in the left-side chop; $r = 0.73$) can easily be determined. Since these y values both refer, though in different ways, to the joint slaughter value of left-side ham + shoulder + chop it may be stated that by means of the ultrasonic measuring method which gives index A_4 the slaughter value of live higs can be assessed with an accuracy sufficient for practical purposes. On the basis of the above this method of measuring is worth using on large-scale farms, primarily in the farm's own performance tests.

On the basis of the experimental results the following conclusions can be drawn:

a) By means of ultrasonic measurements on live hogs the amount of bony meat and lard can be reliably assessed in Hungarian white porkers, as was to be expected on the basis of examinations performed abroad with other breeds ($r = 0.70$—0.77).

b) In the case of ultrasonic measuring the accuracy of the measurements, and thus the reliability of the assessment, can be improved considerably when the measurements are made on animals fixed in a stall;

c) Of the dependent variables which refer to the slaughter value of the more valuable parts of the body (ham, withers, chop), the amount of bony meat contained in the ham can be most accurately assessed by ultrasound as a percentage of the total weight of the ham (y_4; $r = 0.69$), in the shoulder by the weight ratio of bony meat to hide + lard (y_{14}; $r = 0.76$) and in the chop by the weight ratio of bony meat to hide + lard (y_{17}; $r = 0.77$); furthermore, of the dependent variables which characterize the combined slaughter value of ham + shoulder + chop, the total weight of hide + lard in these parts of the body (y_6; $r = 0.78$), the ratio of the total weight of bony meat to hide + lard (y_7; $r = 0.77$), the total amount of lard expressed as a percentage of the live weight (y_9; $r = 0.77$) and the total amount of lard expressed as a percentage of the slaughter weight (y_{11}; $r = 0.77$) can be best assessed by ultrasonic measurements.

d) The correlation values obtained by means of ultrasonic measurements made on half-carcasses after 24 hours of cold storage do not justify the application of this method in practice ($r = 0.45$—0.62).

e) In the case of ultrasonic measuring the closest correlation ($r = 0.68$—0.78) was given by values determined at index A_{29} (thickness of lard measured with ultrasound on withers + back + loins + 2×sides + ham_1 + ham_2 + ham_3 + shoulder_1 : 9) with 12 of the 17 dependent variables. Of the simpler indices containing fewer points of measurement index A_4 (average thickness of dorsal fat) gave correlation values (for 5 of the dependent variables the value of r was greater than 0.71) which indicate that this index is also suitable for the assessment of the slaughter value.

*

Prepared at the Animal Husbandry Research Institute, Herceghalom.

A. Antal, E. Bogdán, I. Gelei, H. Paschke

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EXAMINATION OF CHOPPING PROCESS IN SELF-PROPELLED FORAGE HARVESTERS

Mathematical analysis of distribution of cut length

The mathematical description of the distribution of chopped forage as an end-product of self-propelled forage harvesters and the definition of working quality based on these parameters has not yet been solved. The main problem is that the histograms based on the samples from the mass of cut material are unilateral and cover a range of longitudinal sizes. Thus the expected empirical value and the empirical dispersion give only approximate information about the longitudinal size of the chopped material and the homogeneity of the size. In machine testing practice it is a generally accepted recommendation that the length of 80% of the samples should not exceed 20 mm and any sample should not contain cuts longer than 100 mm.

However this recommendation does not sufficiently represent the quality of the work.

Today, when the conservation of food-stuffs by making silage is becoming more and more important, animal husbandry experts raise increased quality requirements as regards the size distribution of chopped maize. Thus it is reasonable to study the possibility of a better determination of the typical longitudinal sizes around which the cut particles are grouped, i.e. the homogeneity of the end-product. These factors play a very important role in the definition of volume capacity, the condition of lactic-acid fermentation, the storability, the volume weight of forage, the mechanization and automation of exploitation and the portioning of silage, etc. Furthermore, the parameters of length distribution influence the value and usability of harvested fodders to a considerable extent.

The further analysis of work-quality information is also useful for technical experts, because it gives new methods and their energy-saving operation. It makes it possible to study the most important constructional elements more thoroughly and to determine the optimum machine-loading based on energetical parameters in order to achieve the extensive functional testing of the cutting mechanism.

In order to describe the density function a new method has been applied in this study. It is assumed that the distribution of the chopped mass of material is the mixture of three

distributions. According to this principle the end-product comes into existence as the summing result of these three main effects. If it is possible to separate these effects in the end-product, together with the factors which cause them, the working quality may be improved by the help of machine design or operation conditions.

At the same time the length distribution of the cut can be determined more exactly by giving new parameters. It is also assumed that within the density function the effect of two parameters is substantial, i.e. that of the variety of maize plant (characterized by size of stem, leaf and cob) and that of the material flow, and the compacting and chopping processes taking place in forage harvesters. During the analysis of the density function an attempt was made to attain two main objectives, firstly to separate and define distributions, the presence of which can be assumed in these two categories, and secondly to approach the empirical density functions as closely as possible, i.e. to use them to describe the end-product clearly.

The hypotheses regarding the component distributions are as follows.

1. In the case of a separate examination of the cutterhead, assuming that the division of the chopping knives on the drum surface is geometrically exact, the theoretical length of the sample (M_0) can be determined from the known relationship, if the material flow (feeding) is steady, if the cross-section of the particles is homogeneous, their length is infinite and the speed of rotation of the drum is constant (ω = const.). In practice, as a consequence of changes in the above-mentioned parameters the silage length is a probability variable, changing around M_0 according to a normal distribution. This assumption is motivated especially by the fact that the elements of the feeding mechanism are in friction contact with the green product and the normal distribution of the flowing speed belonging to the given parameters has been proved in many cases of friction type movement transmissions.

2. The stem, cob and leaf of the maize plant are of limited length (L_i). From this fact it follows that in the course of the first and last cutting operation into pieces of so-called full-cut, "broken" pieces are produced, the size of which is L_i—n. M_0. (In this conception it was neglected that M_0 should be defined in a size range depending on its S_0 dispersion and in this way the number of broken pieces is an indirect function of S_0 characterizing the work of the drum. Another simplification is to assume L_i as a constant parameter, because the cutting length is also a probability variable, determined by the biological variability, which changes around different average values for the cob, stem and leaf.) The length of the individual particles can change between zero and M_0. Between these size limits the probability of any length of chopped particle arising is equal. Thus this component can be defined as a probability variable of equal distribution.

The dust-type fraction, the share of which does not reach 1% of the total chop weight, and which contains particles in the length range from several μ up to about 2 mm, i.e. shorter than the gap between the standing and moving knives, was neglected; the volume of this fraction is very small and its formation does not play a significant role in the chopping process; it arises in connection with the blowing out after chopping, with the material handling and manipulation of the samples, and finally with the drying of the material. The neglect of this fraction is important to avoid the deforming effect on the histogram when the size of the "dust" fraction is large.

3. With the help of the two previous distributions the empirical density function cannot be approximated sufficiently. There is a need for a mathematical-statistical description of the appearance which represents the occurrence of chop particles longer than the theoretical silage length to a greater extent than the normal size distribution determined by the cutting mechanism.

As regards this third distribution component an unambiguous hypothesis cannot be given, as the occurrence of longer particles depends upon the biological construction of the plant (positioning of the leaves and cobs on the stem, moisture content, etc.), the suitable or

inappropriate operation of the feeding and handling elements on the machines, the density of the plant stock and the thickness of the raw material layer in the feeding unit.

It is also assumed that the distribution of cut particles longer than normal is considerably influenced by the flowing direction of the raw material particles in the cutting gap. Firstly the change in the chop-length caused by a tilting of the flowing material (stems, leaves and cobs) is examined.

The symbols used in Fig. 1 are as follows:

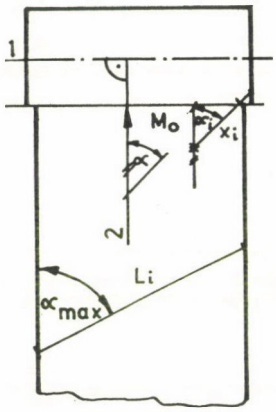

Fig. 1. Schematic diagram for interpretation of increase in chop-length arising as an effect of tilting the particles (1 = shaft of cutting drum, 2 = direction of material flow)

x — length of chop
M_0 — theoretical length of chop
α — angle of tilting compared with straigth-line moving direction
α_{max} — angle of maximum tilting of particles, determined by length of plant (L_i) and width of feeding gap

$$\cos \alpha = \frac{M_0}{x}; \qquad \alpha = \text{arc cos} \frac{M_0}{x}.$$

The distribution function of α:

$$F(\alpha_i) = P(\alpha < \alpha_i) = \int_{-\infty}^{\alpha_i} f(\alpha) d\alpha.$$

The distribution function of x:

$$E(x_i) = P(x < x_i) = \int_{-\infty}^{x_i} e(x) dx$$

where f and e are density functions.

The distribution of x, when the distribution of α is known, can be determined as follows: (α) is increasing continuously, as

$$x = \frac{M_0}{\cos \alpha}, \qquad \text{(Fig. 2.)}$$

$$P(x < x_1) = P(-\alpha_i < \alpha < \alpha_i)$$

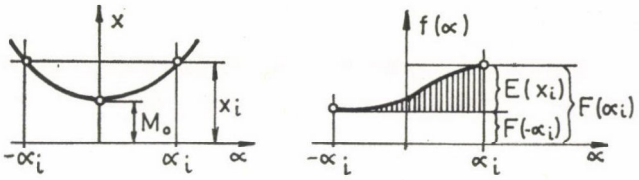

Fig. 2. Diagrams for interpretation of relationship between tilting angle (α) and chop-length (x)

where:

$$x_i = \frac{M_0}{\cos \alpha_i} \quad \text{and} \quad \alpha_i = \arccos \frac{M_0}{x_i} .$$

Thus:

$$E(x_i) = F(\alpha_i) - F(-\alpha_i) = \int_{-\alpha_i}^{+\alpha_i} f(\alpha)\, d\alpha = \int_{-\arccos \frac{M_0}{x_i}}^{\arccos \frac{M_0}{x_i}} f(\alpha)\, d\alpha .$$

After derivation:

$$E'(x_i) = e(x_i) = f(\alpha_i) \frac{M_0}{x_i^2 \sqrt{1 - \frac{M_0}{x_i^2}}} + f(-\alpha_i) \frac{M_0}{x_i^2 \sqrt{1 - \frac{M_0^2}{x_i^2}}} = M_0 \frac{f(\alpha_i) + f(-\alpha_i)}{x_i \sqrt{x_i^2 - M_0^2}}$$

where:

$$M_0 \leq x_i \leq \frac{M_0}{\cos \alpha_{\max}} .$$

If the distribution of α is normal (Fig. 3) then:

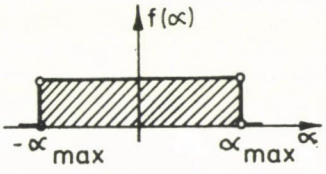

Fig. 3. The assumed distribution function of tilting angle (α)

$$f(\alpha) = \frac{1}{2\alpha_{\max}}$$

as the area under the distribution function is $T = 1$ and its range is $2\alpha_{\max}$.

The density function of the x variable is:

$$e(x) = \frac{M_0}{\alpha_{\max} x \sqrt{x^2 - M_0^2}} .$$

The extreme value of the $e(x)$ function is placed at $x = M_0$, and if x increases, $e(x)$ decreases down to zero (Fig. 4).

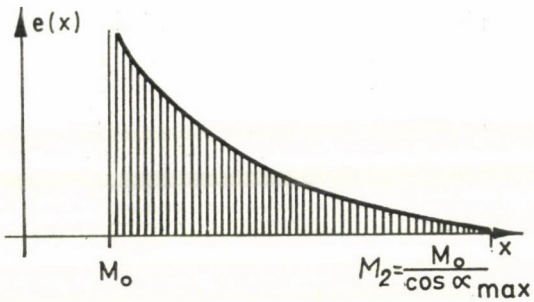

Fig. 4. The assumed density function of chop-length (x)

If the distribution of α is not normal, but falls into a range distant from zero with a continually smaller probability (as can be assumed in the case of a reasonable design of the feeding units and due to the smaller friction resistance around the symmetry line of the feeding gap, which means that the faster moving plants exert a directing effect), then $f(\alpha)$ decreases symmetrically, moving off from zero point, and causes the slope of $e(x)$ to increase. However, at the point $x = M_0$ the singularity of the function still remains, because the area under the density function, as an improprious integral, can only be 1.

Insisting on the earlier hypothesis, i.e. that the characteristic of distribution is influenced, basically, by the tilting of the plants, the approximate density function for the ratio of oversized particles can be given in a simpler form.

Developing the first three members of the Taylor series for the density function:

$$e(x) = \frac{M_0}{\alpha_{max}\, x \sqrt{x^2 - M_0^2}}$$

at the point $M_2 = \dfrac{M_0}{\cos \alpha_{max}}$

we obtain:

$$e(x) = f(M_2) + \frac{f'(M_2)}{1!}(x - M_2) + \frac{f''(M_2)}{2!}(x - M_2)^2 .$$

In this equation $f(M_2) \approx 0$, because the probability that chops longer than the maximum length, determined by the dimensions and by α_{max}, will arise is minimum. It can also be assumed that

$$\frac{f'(M_2)}{1!} \approx 0$$

as the curve is approaching the $x-$ axis and its tangent at point M_2 is approximately parallel with it.

Based on the above-mentioned concepts the density function characterizing the distribution of the oversized ratio can be written as follows:

$$e(x) = a(x - M_2)^2$$

where:

$$a = \frac{f''(M_2)}{2!} .$$

Thus the third component is a parabolic density function distribution giving a good fit to the extended branch of the empirical density function in the longer size range.

The parameters of the three distributions and their ratio in the end-product — assuming it to be a mixture — were defined from the stipulation that the sum of the absolute deviations between values measured at discrete points of two functions, determined from the approaching density function by calculation and based on the empirical density function, should be minimum.

For the approximation in the mixture of separated masses interaction also arises. This effect does not come into existence in connection with the first (rate of broken fragments) and the third mass (rate of over-sized particles) as the occurrence of elements of the two masses mutually precludes each other.

The cross-effect to be found in the size-rate under the expected value of the evenly distributed broken fragments and the "full-cut" normally distributed rate can be incorporated into the uniform distribution without any essential change in its character. Similarly, we can also follow the same method by neglecting the cross-effect over the expected values of the size elements between the over-sized and "full-cut" rates.

In the interest of examining the cross-effects, the joint distribution function of probability variables should doubtless be taken into consideration, but this would require long theoretical considerations which are not reasonable because of the lack of more exact data.

So, the simplified solution of the following problem — as is proved later — produced, in spite of the omissions, a result which gives a more exact description, containing new information, of the cut green mass issuing from forage harvesters according to a longitudinal size distribution.

The problem can be explained graphically according to the diagram in Fig. 5.

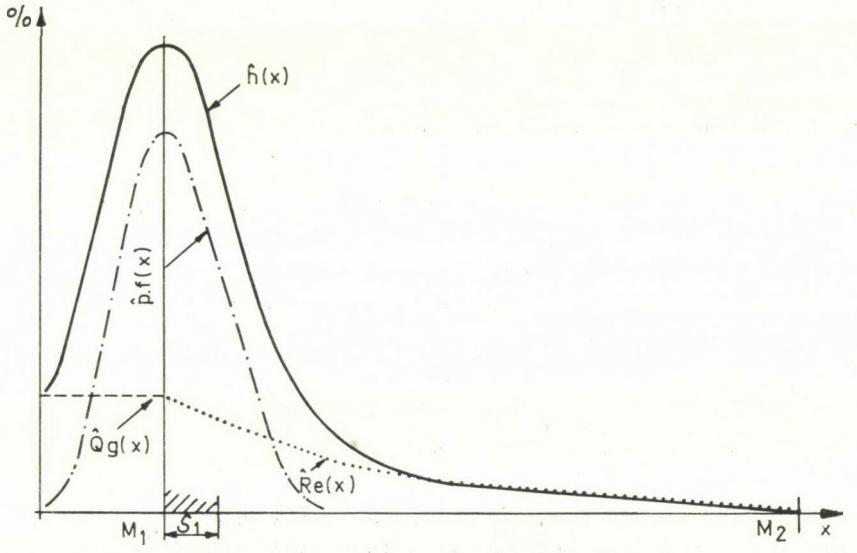

Fig. 5. The assumed components of empirical density function

The most important parameters and data are as follows:

1. The $f(x)$ normal distribution: M_1; S_1 and $\hat{P}$

where: M_1 — expected value,
S_1 — deviation, and
$\hat{P}$ — ratio of well cut particles.

2. The $g(x)$ equal distribution: M_1 and $\hat{Q}$

where: M_1 — parameter of equal distribution
$\hat{Q}$ — ratio of dust fraction.

3. The $e(x)$ parabolic density function: M_2 and $\hat{R}$

where: M_2 — the zero point of the parabolic density function distribution
$\hat{R}$ — ratio of oversized pieces.

Let the length of chop ($\hat{\mu}$) be a probability variable, the density function of which is $h(\hat{x})$. The estimation of the latter is then a histogram based on the sample. Assuming that the mass of material is a mixture of the above-mentioned three distributions, their density functions are:

$$_f(x), \quad g(x) \quad \text{and} \quad e(x)$$

and their proportions in the mixture are:

$$\hat{P}, \hat{Q} \text{ and } \hat{R} \text{ respectively.}$$

It is obvious that $\hat{P} + \hat{Q} + \hat{R} = 1$, i.e. 100%. Further on it is also assumed that the functions $\hat{Q} \cdot g(x)$ and $\hat{R} \cdot e(x)$ are connected to each other continuously, i.e. their values at point M_1 are equal. This is motivated partly by the fact that the empirical density function around point M_1 is approximately a symmetrical concave from below, it does not contain steep decreasing sections and thus it cannot be approximated by component distributions. On the other hand, by neglecting this assumption better results could not be obtained from the point of view of mathematical analysis.

The combined density function is:

$$\hat{h}(x) = \hat{P} \cdot f(x) + \hat{Q} \cdot g(x) + \hat{R} \cdot e(x) .$$

The density functions of component distributions are:
No. 1:

$$f(x) = \frac{1}{\sqrt{2\pi} \cdot s_1} \cdot e^{-\frac{(x-M_1)^2}{2s_1^2}} ; 0 \leq x \leq M_2$$

which is the density function of the probability variable of normal distribution (ξ), and the parameters M_1 and S_1.
No. 2:

$$g(x) = \begin{cases} \frac{1}{M_1} & \text{when } 0 \leq x \leq M_1 \\ 0 & \text{elsewhere} \end{cases}$$

which is the density function of the probability variable of normal distribution (η), and its parameter M_1. The necessity of neglecting the dust-fraction was emphasized earlier. However, it is obvious that the interpretation range for silage length starts with 2 mm as the lower limit

instead of zero. In the course of mathematical analysis this assumption is disregarded and the symbol of zero is used further on.
No. 3:

$$e(x) = a \cdot (x - M_2)^2; \qquad M_1 \leq x \leq M_2$$

which is the parabolic density function of the distribution of the probability variable (S) and the parameters M_2 and a, based on the relationship:

$$\int_{M_1}^{M_2} e(x)\, dx = 1.$$

The value of "a" is determined with the help of parameters taken up earlier.

$$a \int_{M_1}^{M_2} (x - M_2)^2\, dx = 1\,;$$

$$a\left[\frac{(x - M_2)^3}{3}\right]_{M_1}^{M_2} = 1\,;$$

$$-a\frac{(M_1 - M_2)^3}{3} = 1\,; \qquad a = \frac{3}{(M_2 - M_1)^3}\,.$$

Breaking down the combined empirical density function the number of unknown parameters of the component distributions defined earlier is six. These are: M_1, M_2, S_1; $\hat{P}$, $\hat{Q}$ and $\hat{R}$.

Using the method of successive approximation for solving the problem, that approximation is considered as an acceptable one for which:

$$\sum_{s=0}^{n-1} \left| N \cdot \int_{x_s}^{x_{s+1}} \hat{h}(x)\, dx - Z_s \right| \text{ is minimum}$$

where: N — the number of samples
Z_s — the number of samples in the range $(x_s; x_{s+1})$

Otherwise, those parameters are considered acceptable ones where the $\hat{h}(x)$ density function is best approached by the empirical density function.

The program looks for a solution, i.e. for parameters of three component distributions, by changing four independent variables ($\hat{R}$, M_1, M_2 and S_1) simultaneously till the sum of the absolute values of the given deviations for the total empirical density function range becomes minimum.

At this point the computer writes out the computed parameters and the sum of the absolute values of deviations for 20 size classes, and outlines the density function of three component distributions and the combined approximate density function.

The acceptability of the approximation is illustrated in Fig. 6 where the histogram, empirical density function and approximation function of the Hungarian-made HURRIKAN (AS-1,8) are demonstrated. The diagrams of component functions are also given in Fig. 6.

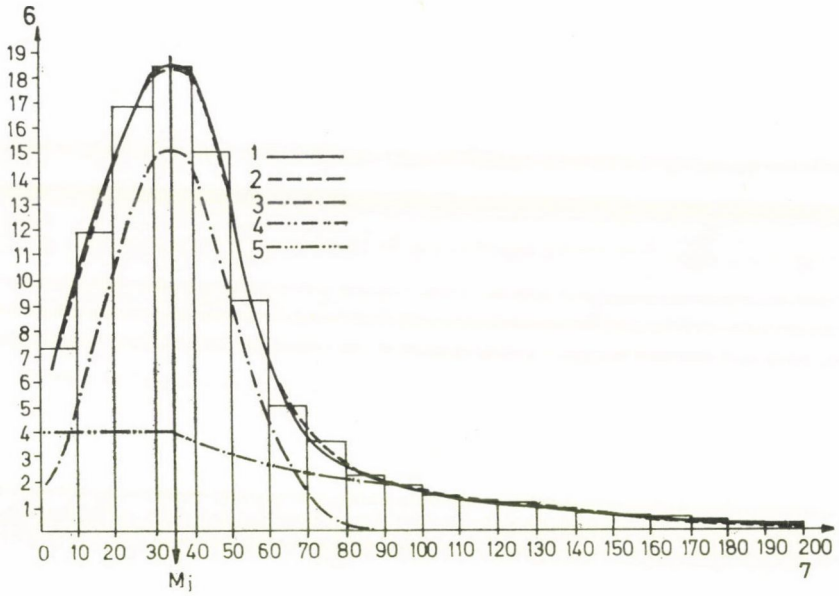

Fig. 6. Chop quality analysis for HURRIKAN (AS-1,8) forage harvester as a comparison between results of mathematical calculations and the components of empirical density function (1 = Empirical density function, 2 = Approximate density function, 3 = Component of normal distribution, 4 = Component of equal distribution, 5 = Parabolic component of density function, 6 = Occurrence [%], 7 = Chop-length [mm])

Conclusions from results of mathematical analysis

1. Based on Fig. 6 it can be established that the approximate function combined from three components follows the empirical distribution very well. This means that the solution is mathematically perfect. However, it can be seen from the diagrams that the component density function of normal distribution as the final result also stretches into the negative part in practice. Therefore the representation of this range has been omitted and $f(x)$ is taken as zero in the range $x \leq 0$. The degree of error caused by this assumption is questionable, as the function is asymmetrical in the range $x < 0$, where it has physical meaning, and therefore the parameters M_1, S_1 and $\hat{Q}$, used as numerical values later, should be corrected.

Deviations originating from the definition of the density function of normal distribution do exist, but only to a small extent, as the valid range of the function is $(-\infty < x < +\infty)$ and that of the chop-length is $(0 < x < \infty)$, practically M_2. The error could be practically eliminated if this component were determined as a curtailed normal distribution instead of a normal distribution; however in this case, because of the introduction of new parameters, the method of approximation would become very complicated and the possibility of errors arising would increase. Therefore the method of calculation has not been changed but further assumptions have been made.

The result of the mathematical analysis for chop-length can be accepted for practical use, if:

$$M_1 \geq 2S_1$$

i.e. if the distribution of the normal component is symmetrical at least in the range:

$$\pm 2S_1$$

for M_1. In this way some 95.4% of the data for chop-length can be used. (When $M_1 \geq 2S_1$ this is ~99.7%). It follows from previous findings that this method can only be used for the analysis of the chop-length distribution of forage harvesters which make so-called "exact" chops. In our example this condition has been realized for the AS-1,8 type forage harvester (values for $M_1 > 2S_1$ are $33.13 > 2 \times 16.10$) and the results obtained are suitable for the drawing of practical conclusions.

2. The main objective was to draw conclusions for technical features of self-propelled forage harvesters. Therefore the most labour requiring data-processing method was chosen. In this way numerous probability variables were eliminated which originated from the biological construction and variability of the plants, these being significant in the weight distribution of the cut-length, thus making it difficult to analyse the operation characters of the machine.

However, in practice, instead of piece distribution of chop-length, the weight distribution is often given. The relation between these two parameters is based on the distribution of

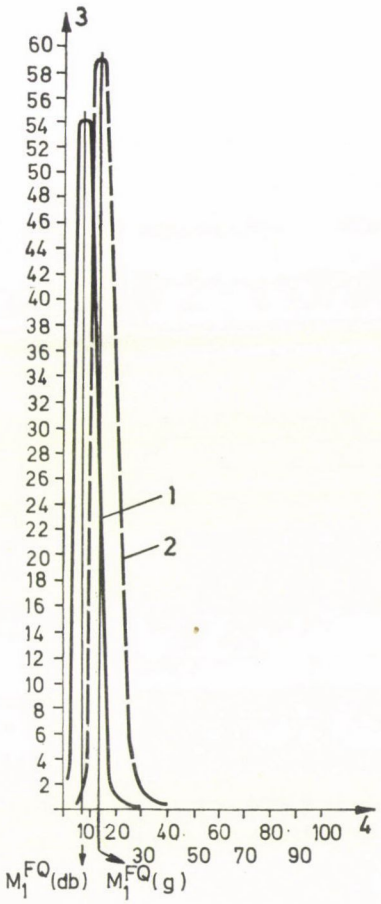

Fig. 7. Comparison of empirical density functions based on ratio of particles (%) and weight ratio (%) for FIELD QUEEN forage harvester (1 = Number of particles [%], 2 = Weight [%], 3 = Occurrence [%], 4 = Chop-length [mm])

the plant cross-section, which can be taken as a constant factor for a given maturity and plant variety and is obviously independent of the design and technical state of the machine.

The empirical density functions originating from these two data-processing methods are quite similar for "exact" forage harvesters and their typical circumstances approach each other, as is proved by the cut-length distribution of the FIELD QUEEN type (HESSTON) self-propelled, high capacity (80 Mp/h) forage harvester, which can be seen in Fig. 7.

Utilization of results of mathematical analysis for comparative test of forage harvesters

To examine the development of forage harvesters according to the quality of work during the past decade the parameters given by mathematical analysis of chop-length can be used.

The FIELD QUEEN type self-propelled forage harvester (produced in 1974) was used for comparative evaluation. The production data and characteristics of samples and machines are given in Table 1, while the parameters of component distributions and distribution functions and the mixing ratios of the components are demonstrated in Table 2. In the case of the HURRIKAN (AS-1,8) type forage harvester 61% of the chop mass corresponded to the technical parameters of the machine, while in the case of the FIELD QUEEN forage harvester this parameter proved to be 94.5%, which could be taken as a properly cut ratio ($\hat{P}$).

Table 1

Technical and operational data for HURRIKAN (AS-1.8) '64 and FIELD QUEEN '74 forage harvesters

Testing	Types	HURRIKAN (AS-1.8)	FIELD QUEEN
	place, date	"Táncsics" coop. farm, Mezőhék Aug. 1965	State farm, Szombathely Sept. 1974
Produce	Plant	maize	maize
	variety	Mv-1 (Martonvásár)	Sx-39 (USA)
	plant population (ha)	43,000	80,000
	yield (ton/ha)	35	55
	average height (cm)	180	250
	average m.c. (%)	72	75
	maturity state	full ripeness	full ripeness
Machine	operation system	tractor driven	self-propelled
	engine (HP)	90	210
	loading control	manual, according to gearstages of tractor	continuous, hydrostatically controlled
	average loading (Mp/h)	12	59
	working width (m)	1.8	3 rows of plant
	type of cutter-bar	cutting table	row type
	type of cutterhead	self-throwing drum	feeding onto blowing fan
	drum diameter, D (mm)	550	630
	length, L (mm)	1,800	580
	r.p.m.	1,210	1,250
	theoretical cut-length (mm)	33	7.94
Chop	No. of samples	80×50 g	10×50 g
	No. of sample elements	12,571	20,122
	No. of sections of histograms	20	20
	range (mm)	10	5

Table 2

Evaluation of feeding and cutting units of HURRIKAN (AS-1.8) and FIELD QUEEN forage harvesters

Type		Distribution: Density function	Mixing: parameters (mm)		ratio (%)	HURRIKAN (AS-1.8)	FIELD QUEEN
Empirical			Fig. 8./a)	$\hat{M}_e$		44.74	8.80
				$\hat{S}_e$		33.48	4.61
				theoretical chop-length M_0		33.00	7.94
Mixture consisting of three components		$\hat{h}(x) = \hat{P}f(x) + \hat{Q}q(x) + \hat{R}e(x);\quad 0 < x < M_2$	Fig. 8./b)	average error of approximation for one size range, Δ (%)		0.182	0.032
					$\hat{P} + \hat{Q} + \hat{R}$	100	100
Components	Normal (properly cut)	$f(x) = \frac{1}{\sqrt{2\pi \cdot S_1}} \cdot e^{-\frac{(x - M_1)^2}{2S_1^2}};\quad 0 < x < M_2$	Fig. 8./c)	M_1		33.13	8.41
				S_1		16.10	3.13
					$\hat{P}$	61.00	94.50
	Equal (dust-fraction)	$g(x) = \frac{1}{M_1};\ 0 < x < M_1$; 0; elsewhere	Fig. 8./d)	M_1		33.13	8.41
					$\hat{Q}$	13.00	2.10
	Parabolic density function (oversized)	$e(x) = \begin{cases} a(x - M_2)^2; & M_1 < x < M_2 \\ 0; & \text{elsewhere} \end{cases}$, $a = \frac{3}{(M_2 - M_1)^3}$	Fig. 8./e)	M_2		227.86	51.20
					$\hat{R}$	26.00	3.50

If the machine is operated under field conditions the expected value of this normal distribution ratio M_1 should be equal to the theoretical chop-length. During the experiments values of $M_0 = 33.0$ mm and $M_1 = 33.13$ were measured, which means that

$$M_0 \cong M_1 .$$

The testing recommendation prescribes that 80% of the chop-length should be under 20 mm and the maximum length should not exceed 100 mm. In the case of the HURRIKAN (AS-1,8), $M_2 = 227.86 > 100$, i.e. the second condition was not realized. With the help of an $h(\hat{x})$ density function the fulfilment of the first condition is now examined.

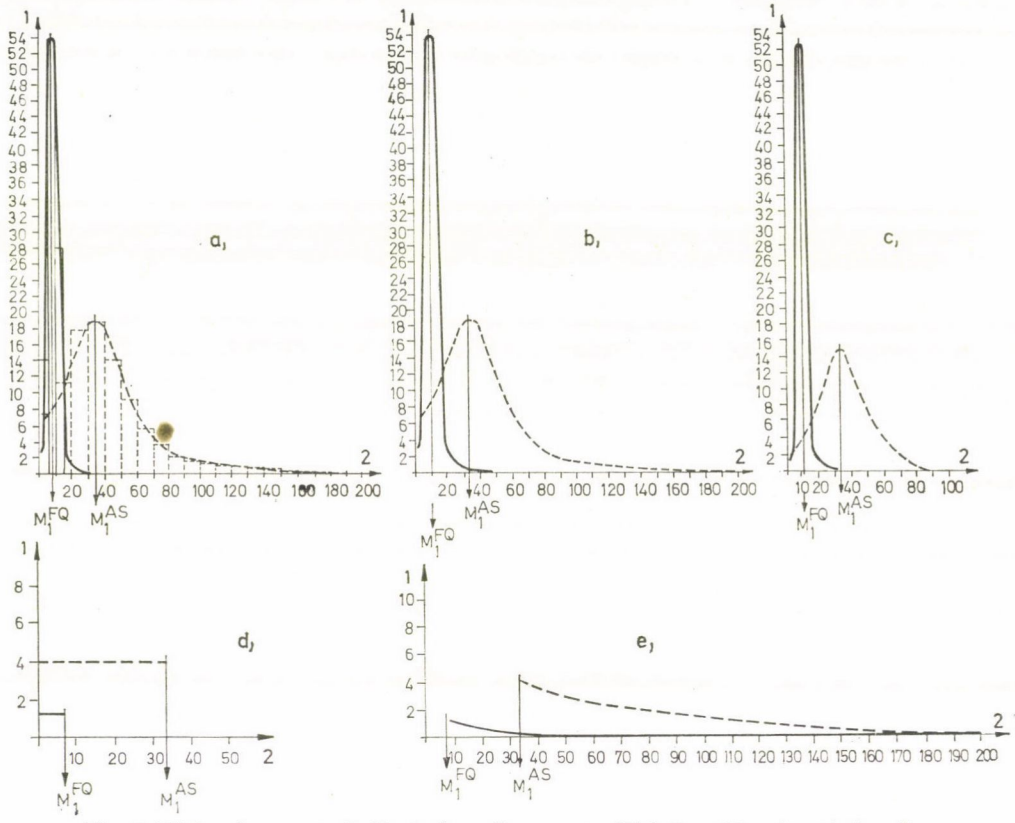

Fig. 8. With reference to Table 2. (1 = Occurrence [%], 2 = Chop-length [mm])

If the quantity of material in the (0; 20) size-range is Ω, and $20 \leq M_1$ then

$$\Omega = \frac{\hat{P}}{\sqrt{2\pi} \cdot s_1} \int_0^{20} e^{-\frac{(x-M_1)^2}{2s_1^2}} \, dx + \int_0^{20} \frac{\hat{Q}}{M_1} \, dx .$$

After solving this differential equation and substituting the parameters concerned:

$$\Omega = 0.193 \quad \text{i.e.} \quad 19.3\% .$$

The ratio of chop-particles under 20 mm is approximately 20% for the HURRIKAN (AS-1,8) forage harvester (produced in 1964).

The theoretical chop-length for the FIELD QUEEN machine was $M_0 = 7.94$ mm. The expected value of the properly cut fraction was $M_1 = 8.41$ mm, i.e. practically:

$$M_0 \cong M_1 .$$

The factors $M_2 = 51.2 < 100$ mm and 94.5% of the chop mass were prescribed by the normal distribution of chop-length, and 99.7% of the material fall into the $6S_1$ (0; 18.78) size-range ($S_1 = 3.13$ mm and $6S_1 = 18.78$ mm). This means that $\Omega > 0.945 \times 0.997 = 0.9422$, i.e. at least 94.22% of the chop mass belongs in the length range under 20 mm. (Taking into consideration the dust fraction and the size-range between 18.78 and 20 mm this value increases further.)

Based on these figures it can be assumed that the machine is able to satify the standard prescriptions for a chop-length of 11.11 mm too.

In the end-product of the HURRIKAN (AS-1,8) machine, considered as a mixture, the ratio of over-sized material was considerable: 26%. A considerable part of the material was not chopped according to the kinematical conditions determined by the feeding and chopping units, or could not be chopped at all, since in the chopping chamber the spreading of the material is not equal and the material flow is uneven. Under these circumstances the feeding elements are not able to direct the particles, especially the leaves and cobs, perpendicular to the shaft of the drum in an even manner. At the same time the FIELD QUEEN machine, where the ratio of over-sized material was only 3.5% showed, a favourable quality of work in consequence of employing a suitable cutting mechanism, a restricted feeding unit and double upper and lower feeding elements.

The deviation for a well chopped ratio of material was $S_1 = 16.10$ mm for the HURRIKAN (AS-1,8) machine, while the relative deviation (V) was 49%.

The probable reason for the large deviation was that the relative velocity ratio of the different constructional elements is changeable and the velocity of certain parts of the plants also changes because of the uncertain friction of the plants. (The chop-length is influenced by small fluctuations in the r.p.m. of the tractor engine. If, for example, the power take off 8 haft rotates with a smaller velocity a longer time passes between two cuts, but the forward movement of the material is also slower, so the length of the chops remains constant.)

In my opinion the deviation is caused by the following factors:

— the elastic and absolute belt slip due to the dynamic effect of the elastic driving elements (the upper feeding roll and drum drive),

— the uneven material flow through the feeding unit, which is the same width as the cutting mechanism,

— the small inertia moment of the cutting drum (which means that the drum is unable to overcome the peak loadings which occur periodically).

The solution is to apply more highly developed driving elements (teeth belts or chains), as well as cutting drums of larger diameter and thus of higher inertia moment.

For the FIELD QUEEN cutting mechanism values of $S_1 = 3.13$ mm and $V = 37\%$ were found. This means that the deviation of the properly cut ratio is small, the velocity ratio of constructional elements does not change during operation and the inertia moment of the drum is suitable; with the help of the velocity-meter in the hydro-static driving unit the operator can set the loading of the machine to optimum at any time, and the peripheral velocity fluctuation of the knives can be eliminated.

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Prepared at the Agricultural Machinery Institute, Faculty of Agricultural Engineering, University of Agricultural Sciences, Gödöllő.

P. SZENDRŐ

EFFECT OF DIFFERENT LIGHT INTENSITIES ON THE ANATOMICAL CHARACTERISTICS OF THE LEAVES OF PIMPINELLA ANISUM L.

Zalenski (SINNOT 1960) observed that the structure of the leaf changes according to the level of insertion on the shoot. The leaves show more and more xeromorphic features with increasing distance from the root, namely, smaller cells, less intercellular space, smaller in size but higher the number of stomata per unit area, thick walled epidermis and more highly developed palisade and mechanical tissues. These observations, known as Zalenski's law, were later verified by many workers in numerous plant species. Many later publications are available which indicate that the structure of the leaves, particularly the ratio between spongy and palisade parenchyma and the proportion of palisade tissue, is correlated with the intensity of the light available to the plants (SHIELDS 1952, FEKETE—SZUJKÓ-LACZA 1973).

SCHÜRMANN (1959) has pointed out that water plays an important role in the initiation of stomata. KNECHT—O'LEARY (1972) has experimentally verified the relationship between the number of stomata and the light intensity. (In natural conditions the young leaves of the stem receive more light than the old ones during the period of differentiation.)

WEAVER—CLEMENTS (1938) are of the opinion that the upper leaves developed later do not have more stomata per area than the lower ones. The cells and stomata are smaller in the upper leaves than in the lower ones.

From a physiological point of view, Sestak and Ticha (TICHA 1968) noted changes in the metabolic activities of the leaves from base to apex. MARÓTI (1976) pointed out that in the case of fully developed leaves, the proportion of photosystem I in palisade parenchyma is equal to that of spongy palenchyma, but that of photosystem II is higher. A quantitative analysis of Zalenski's law was first made in many anatomical characters by SZUJKÓ-LACZA (1974) in *Pimpinella anisum* L. grown under natural conditions. Among others she noted that different characters of the leaves change in opposite directions.

In the present paper, the authors have made a comparative study of the change in leaf structure in plants grown under natural conditions and those treated under different light intensities, in order to discover the connection between xeromorphy and light intensity.

The plants were grown under natural conditions in the Soroksár horticultural garden near Budapest in 1972—1973. For the light treatment, the plants were raised in a phytotron at Szeged on 21st May 1973 and studies were made the same year on 28th June. In one chamber of the phytotron the plants were treated under a light intensity of 13,000 lux (52,000 erg/cm^2/sec) and in the other under 5,000 lux (20,000 erg/cm^2/sec) using light from F29 neon tubes made in Hungary (Egyesült Izzó). The illumination time was 16 hours per day. The temperature was maintained in both chambers at 15°C at night and 25°C in the day-time. The relative humidity of the chambers was kept at 26—64%. A detailed description of the phytotron used has been given by HORVÁTH (1972). The plants were raised from seed in earthen pots. Each pot contained three plants and each chamber three pots. One plant was randomly selected from each pot (a total of three from each chamber). The leaves were collected from the stem seriatim from base to apex. The uppermost part of the leaf, just below the double teeth margin, was selected for section cutting. The selected samples were fixed in Nawashin solution, embedded in paraffin and stained in Toluidine blue, after which 15 μm thick microtome sections were prepared. From each leaf three transverse sections were studied. The mean values of the three sections of each leaf were used as basic data for the calculations, referred to unit area of leaf transverse section (hereafter: per unit area), according to the rule of three. The variance data were analysed as suggested by SVÁB (1967). The anatomical characters are represented in the tables by the abbreviation c. Each character has an index (c_1, c_2, c_3, . . .). The characters c_1, c_2, c_3, c_{10} and c_{11} were multiplied by 100 for calculation. The data c_4—c_9 were referred to unit area of leaf transverse section. (It should be pointed out here that the

thickness of the palisade parenchyma was determined by subtracting the area of spongy parenchyma in the cross section from that of the total area of leaf in the cross section. The palisade tissue includes the xylem of the vascular bundles and a row of transitional cells between the palisade and the spongy parenchyma.)

Tables 1 and 10 contain the data obtained from plants under natural conditions.

Table 1

Mean values of the anatomical characteristics of Pimpinella anisum leaves under natural conditions

Serial No. of leaves	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}
1	1.0859	0.5275	0.5583	13.8	7.0	42.0	31.7	28.3	30.0	0.0726	0.1474
2	1.0693	0.5477	0.5217	18.4	7.2	45.6	38.8	38.5	38.5	0.0554	0.1402
3	1.1288	0.5604	0.5683	19.4	7.4	54.1	39.9	32.5	46.5	0.0520	0.1392
4	1.0416	0.4623	0.4597	19.8	7.6	48.5	35.3	30.5	39.9	0.0511	0.1315
5	0.9005	0.4504	0.4501	22.0	9.2	59.9	31.7	29.9	39.3	0.0461	0.1098
6	0.9894	0.4984	0.4910	19.5	9.0	62.5	29.0	45.6	45.6	0.0544	0.1184
7	0.7653	0.3950	0.3703	20.2	11.4	65.0	41.7	30.7	36.1	0.0500	0.0884
8	1.1773	0.6094	0.5679	24.6	11.2	67.8	35.4	33.4	34.4	0.0409	0.0909
9	0.9110	0.4273	0.4836	24.0	12.9	81.5	44.8	27.1	35.3	0.0444	0.0775
10	0.7573	0.3813	0.3760	27.8	14.1	92.9	59.3	36.0	47.9	0.0366	0.0714

c = anatomical characters; c_1 = area of leaf in mm^2; c_2 = area of spongy parenchyma layer in mm^2; c_3 = area of palisade parenchyma layer in mm^2; c_4 = number of vascular bundles per unit area; c_5 = number of secretory canals per unit area; c_6 = number of epithelial cells per unit area; c_7 = number of stomata on lower side of the spongy parenchyma per unit area; c_8 = number of stomata on upper side of the palisade parenchyma per unit area; c_9 = total number of stomata per unit area; c_{10} = mesophyll area of one transport bundle; c_{11} = mesophyll area of one secretory canal (see also in Tables 2, 3, 4, 7, 8 and 9).

Plants grown under 13,000 lux produced eight leaves per plant, whereas those under 5,000 lux produced six leaves each. The basic data obtained under different conditions are given in Tables 1, 2 and 3. For plants grown under natural conditions, of the three anatomical characters which coincide with those mentioned by Zalenski (SINNOT 1960), the behaviour of c_3 was not unanimously xeromorphic, while characters c_4 and c_7 followed Zalenski's law. In Table 2, the same characters in plants grown under 5,000 lux did not exhibit any unidirectional tendency. For plants grown under 13,000 lux, all three characters followed Zalenski's law, except in the 5th and 6th leaves, which showed relatively low values for c_7. Also some modification was found in the case of c_3, depending on the insertion of the leaves. This result shows that there is also some connection between the light intensity and the xeromorph characters of the leaves.

Table 4 presents the total number of significant differences. It shows the response of the quantitative characters of the leaves to different light intensities. The number of secretory canals and the number of epithelial cells per unit area are more constant than the rest of the

Table 2

Mean values of the anatomical characteristics of Pimpinella anisum leaves grown under a light intensity of 5,000 lux (□)

Serial No. of leaves	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}
1	31.35	10.25	21.09	19.76	28.96	58.73	119.50	37.83	57.73	26.47	3.57
2	15.77	27.20	45.77	25.30	18.50	27.87	58.43	23.53	35.00	16.50	3.72
3	67.99	11.92	38.63	13.73	22.16	41.13	65.00	20.60	31.03	7.45	4.62
4	80.36	15.00	43.95	18.43	29.10	31.97	64.30	16.70	28.73	5.60	3.52
5	77.98	18.77	59.21	16.83	26.25	24.43	61.50	19.80	29.63	5.99	3.93
6	31.03	24.44	24.60	29.00	50.66	72.93	115.97	24.73	49.16	3.92	2.23

(□) The data were multiplied by 100 for calculation.
Tables 2—9, 11 and 12 represent the data collected from plants grown under controlled conditions.

Table 3

Mean values of the anatomical characteristics of Pimpinella anisum leaves grown under a light intensity of 13,000 lux (□)

Serial No. of leaves	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}
1	73.08	16.99	56.67	12.66	20.66	32.70	56.53	9.53	20.53	8.13	5.94
2	110.42	32.91	77.57	14.20	21.46	21.73	35.17	15.06	23.80	7.23	4.06
3	118.90	22.40	64.91	17.56	25.23	24.60	52.07	14.56	24.83	5.82	4.31
4	106.10	17.36	58.35	16.33	22.36	29.43	105.23	23.97	42.50	8.00	4.38
5	106.00	20.56	30.70	14.50	23.83	42.30	60.23	23.60	33.80	7.88	3.72
6	115.12	23.02	67.23	13.33	20.90	23.96	92.53	28.57	44.90	7.60	4.09
7	135.15	23.71	78.40	19.90	31.03	26.50	154.57	50.17	73.97	5.03	3.25
8	40.29	5.70	25.12	33.63	52.03	69.63	400.63	77.90	137.07	3.01	1.93

(□) The data were multiplied by 100 for calculation.

characters under a particular light intensity (Table 4). Microphotographs show the 4th leaf in each case (Figs 1, 2 and 3) and Table 4 indicates that the significant differences in anatomical characters are variable according to the different light treatments. This is clearly evidenced by character c_6 (epithelial cells per unit area of leaves), where the significant differences show a gradual decrease from the natural conditions to the lower light intensity. The area of palisade parenchyma seems to be highly sensitive to light intensity and to react in a reverse direction, i.e. the number of significant differences between the leaf pair increases with a decrease in light intensity.

Leaves treated under 13,000 lux had a higher number of significant differences than those treated under 5,000 lux (Tables 5 and 6). The distribution of significant differences per

Table 4

Total number of characters with significant differences (S.D.) at different levels of significance under different light intensities

Character No.	5,000 lux	13,000 lux	Natural conditions
c_1	0	10	6
c_2	0	11	6
c_3	6	4	3
c_4	0	7	9
c_5	5	12	11
c_6	5	7	20
c_7	0	11	0
c_8	0	16	13
c_9	0	17	16
c_{10}	0	11	7
c_{11}	0	1	0
Total	16	107	91

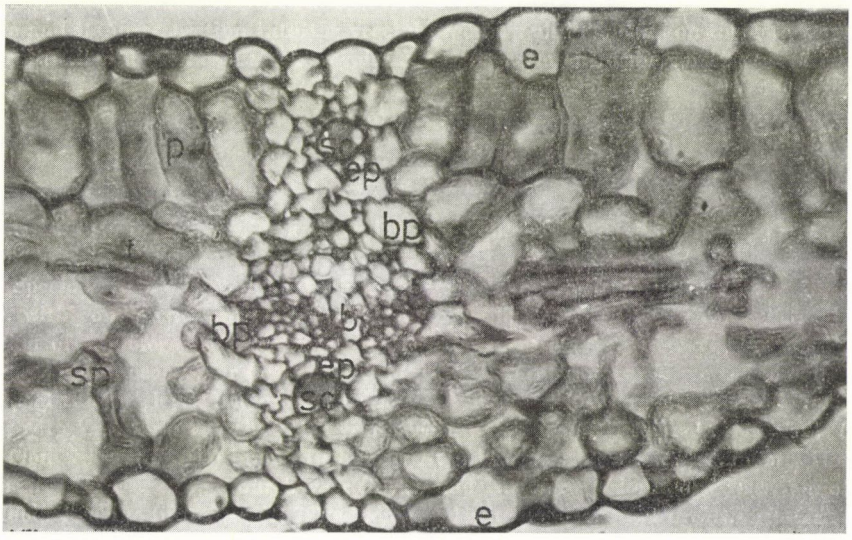

Fig. 1a. Transverse section of *Pimpinella anisum* leaf (natural conditions) 300× (bp = bundle sheath parenchyma; e = epidermis; ep = epithelial cell; p = palisade parenchyma; sc = secretory canal; sp = spongy parenchyma; t = transitional cell; vb = vascular bundle)

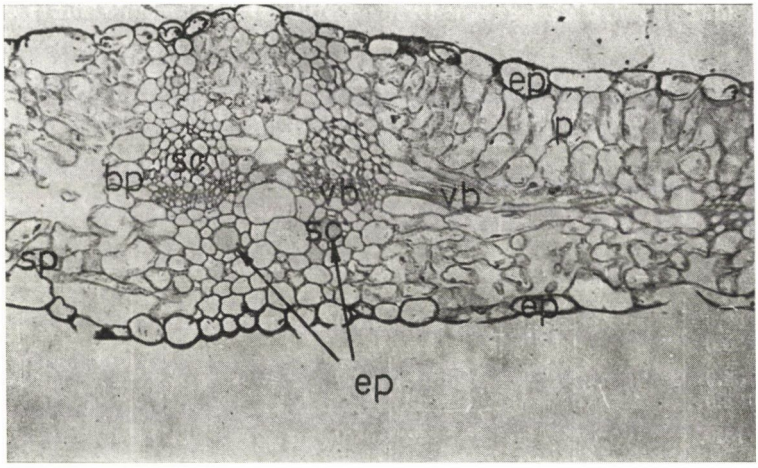

Fig. 1b. Transverse section of *Pimpinella anisum* leaf (natural conditions) 120× (bp = bundle sheath parenchyma; e = epidermis; ep = epithelial cell; p = palisade parenchyma; sc = secretory canal; sp = spongy parenchyma; t = transitional cell; vb = vascular bundle)

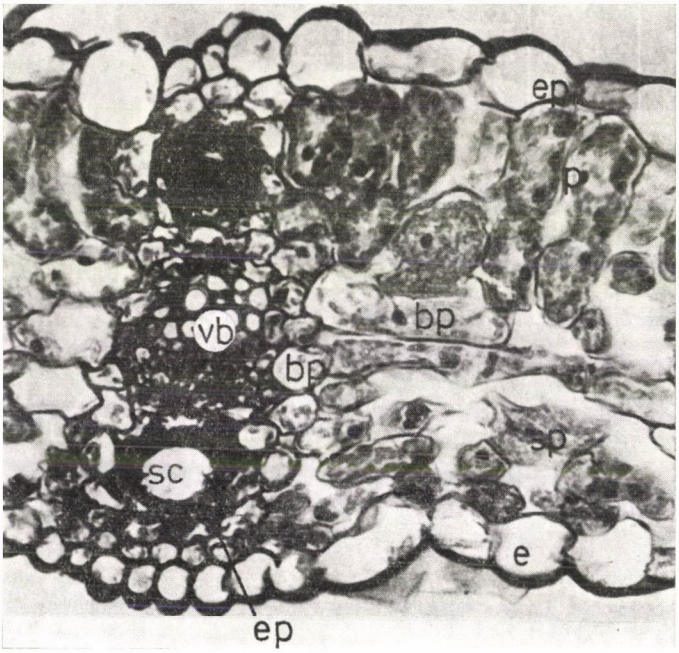

Fig. 2a. Transverse section of *Pimpinella anisum* leaf (13,000 lux) 300× (bp = bundle sheath parenchyma; e = epidermis; ep = epithelial cell; p = palisade parenchyma; sc = secretory canal; sp = spongy parenchyma; t = transitional cell; vb = vascular bundle)

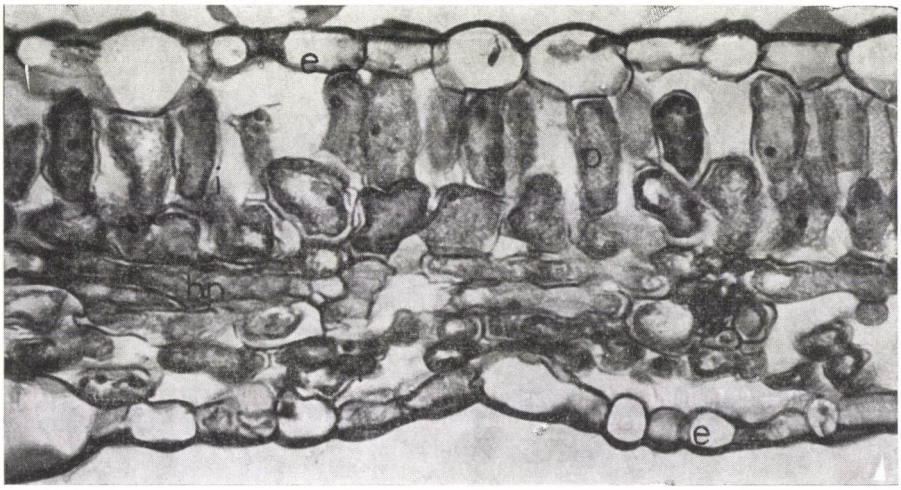

Fig. 2b. Transverse section of *Pimpinella anisum* leaf (13,000 lux) 300× (bp = bundle sheath parenchyma; e = epidermis; ep = epithelial cell; p = palisade parenchyma; sc = secretory canal; sp = spongy parenchyma; t = transitional cell; vb = vascular bundle)

Table 5

Total number of significant differences at different levels of significance according to the serial number of the leaf (5,000 lux)

Serial No. of leaves	5%	1%	0.1%	Total
1				0
2				0
3				0
4	1			1
5	2			2
6	10	3		13
Total	13	3		16

anatomical characteristic under natural conditions is almost the same as that under 13,000 lux. On the other hand only three characters (c_3, c_5 and c_6) show significant differences under 5,000 lux (Tables 6, 7, 8 and 9). The distribution of significant differences between leaf pairs is quite different under the different light treatments. In natural conditions the frequency distribution of significant differences between the leaves follows Zalenski's law (Table 10, column II).

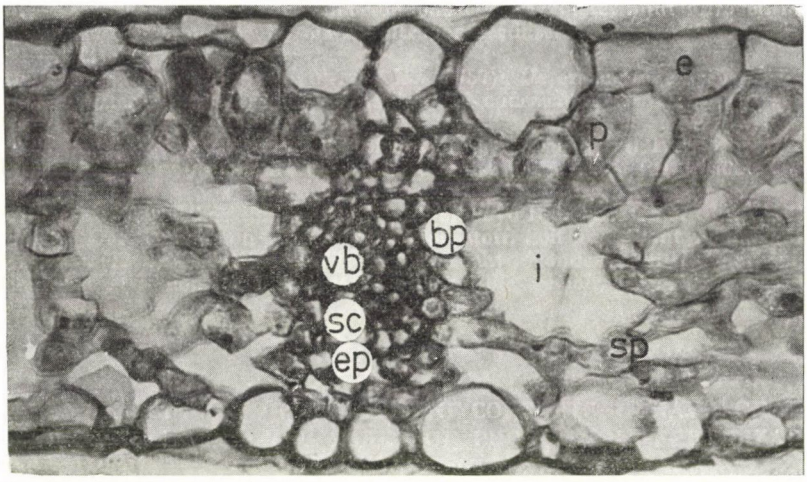

Fig. 3a. Transverse section of *Pimpinella anisum* leaf (5,000 lux) 300× (bp = bundle sheath parenchyma; e = epidermis; ep = epithelial cell; p = palisade parenchyma; sc = secretory canal; sp = spongy parenchyma; t = transitional cell; vb = vascular bundle)

Fig. 3b. Transverse section of *Pimpinella anisum* leaf (5,000 lux) 300× (bp = bundle sheath parenchyma; e = epidermis; ep = epithelial cell; p = palisade parenchyma; sc = secretory canal; sp = spongy parenchyma; t = transitional cell; vb = vascular bundle)

Table 6

Total number of significant differences at different levels of significance according to the serial number of the leaf (13,000 lux)

Serial No. of leaves	5%	1%	0.1%	Total
1				0
2	1	1		2
3	3	0		3
4	2	1		3
5	3	0		3
6	5	1		6
7	8	12	6	26
8	12	23	29	64
Total	34	38	35	167

Table 7

Distribution of S.D. between anatomical characteristics and serial number of leaves under natural conditions (regardless of the serial number of leaves which differ from each other)

No. of characters No. of leaves	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}	Total
1	2	2		4		5		2	5	7		27
2				1	1	4		1	1			8
3	2	2		1	2	2		1	4			14
4				1	2	3		2	1			9
5					2	2		3				7
6				1	2	2		1	2			8
7	1	1	1	1	1	1		1	1			8
8	1	1	2		1	1		1	1			8
9								1	1			2
10												0
Total	6	6	3	9	11	20	0	13	16	7	0	91

Table 8

Distribution of S.D. between anatomical characteristics and serial number of leaves at 5,000 lux (regardless of the serial number of leaves which differ from each other)

No. of characters No. of leaves	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}	Total
1		2		1	1							4
2		1		1	1							3
3		1		1	1							3
4		1		1	1							3
5		1		1	1							3
6												0
Total		6		5	5							16

Under 13,000 lux the number of significant differences between the leaves from the 1st to the 5th are nearly constant or only slightly different. In the case of the 6th and 7th leaves the number of significant differences increases gradually but in the 8th it become to more than twice that in the 7th. This result indicates that the first five leaves show low significant differences compared to those under natural conditions (Table 12, column II). At lower light intensity (5,000 lux) the first four leaves do not show any significant differences, which are restricted to the 5th and the 6th leaves. But these two leaves show unexpectedly high significant differences (Table 11, column II).

Table 9

Distribution of S.D. between anatomical characteristics and serial number of leaves at 13,000 lux (regardless of the serial number of leaves which differ from each other)

No. of characters No. of leaves	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}	Total
1	4	2		1	2	1	2	5	4	3		24
2	1	4	2	1	2	1	2	2	3	1		19
3	1	1		1	1	1	2	2	3	1		13
4	1	1		1	2	1	1	2	2	2		13
5	1	1	1	1	2	1	2	2	2	2		15
6	1	1		1	2	1	1	2	2	2		13
7	1	1	1	1	1	1	1	1	1		1	10
8												0
Total	10	11	4	7	12	7	11	16	17	11	1	107

Table 10

Number of S.D. per leaf pair, i.e. leaf levels under natural conditions (regardless of the nature of the anatomical characteristic). Symbol: I. Total for row; II. Number of S.D. (number of observations in %); III. Total of row and column totals

Serial No. of leaves	1	2	3	4	5	6	7	8	9	I	II	III
1										0	0	27
2										0	0	8
3	2									2	9.1	16
4	2									2	6.1	11
5	4									4	9.1	11
6	1	0	1	2	1					5	9.1	13
7	4	1	4	1	0	1				11	16.7	19
8	3	2	2	2	0	1	3			13	16.9	21
9	4	1	2	1	3	3	0	1		15	17.1	17
10	7	4	5	3	3	3	5	7	2	39	39.4	39
Total	27	8	14	9	7	8	8	8	2	91	113.5	182

Table 11

Number of S.D. per leaf pairs, i.e. leaf levels under a light intensity of 5,000 lux (regardless of the nature of the anatomical characteristic)

No. of leaf	1	2	3	4	5	I	II	III
1						0	0	4
2						0	0	3
3						0	0	3
4						0	0	3
5	2		1			3	4.54	6
6	2	3	2	3	3	13	19.70	13
Total	4	3	3	3	3	16	24.24	32

Symbol: I = Total for row; II = Number of (S.D. number of observations in %); III = Total of row and column totals.

Note: The leaf pairs were constituted as follows: the 1st leaf with the 2nd, the 1st with the 3rd . . . the 2nd with the 3rd, the 2nd with the 4th . . . etc. In Tables 11 and 12 the half matrices show the significant differences between any z_{ij} element, in the ith row (where $i = 1, 2, 3 \ldots$) and in the j-th column (where $j = 1, 2, 3 \ldots$).

According to these results the statement of WEAVER—CLEMENTS (1929) may be true in the case of leaves grown under a light intensity of 13,000 lux but not in those grown at low light intensity. In Tables 4, 8 and 9 the number of secretory canals and the number of epithelial cells per unit area of leaves show fewer significant differences in plants raised at low light intensity than in those grown at high light intensity or under natural conditions. But in every case the plants show some significant difference, so it may be assumed that those characters which show significant differences even at low light intensity are less affected by this factor than the others.

It should be noted that the significant differences under natural conditions (Table 10) are almost constant in the middle leaves and that they go no changing in both direction, i.e. towards the apex and the base. Under controlled conditions the first 4 or 5 leaves do not show a large number of significant differences (Tables 11, 12).

Table 12

Number of S.D. per leaf pairs, i.e. leaf levels under a light intensity of 13,000 lux (regardless of the nature of the anatomical characteristic)

No. of leaf	1	2	3	4	5	6	7	I	II	III
1								0	0	25
2								2	2.27	19
3	2	1						3	3.41	18
4	2	1						3	3.41	18
5	1	1						2	2.27	16
6	3	1	1					5	5.68	18
7	6	4	4	5	5	4		28	31.82	37
8	9	9	10	10	9	9	9	65	73.86	65
Total	25	17	15	15	14	13	9	108	122.72	261

Symbol: I = Total for row; II = Number of S.D. (number of observations in %); III = Total of row and column totals.

Lastly, it may be concluded that the middle leaves should show a more or less constant mean value. This seems to be true from a physiological point of view as well (TICHA 1968). The basal and apical leaves, on the other hand, should give quite variable results, very different from the constant value of the middle portion.

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Prepared at the Botanical Department, Natural History Museum, Budapest; Department of Botany, Serampore College Serampore, West Bengal; Department of Botany, Attila József University, Szeged.

J. SZUJKÓ-LACZA, S. SEN, I. HORVÁTH

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PHYTOCENOLOGICAL STUDIES ON SINGLE-SPECIES GRASSES

Comparative floristic and phytocenological examinations of planted grasses are of importance both theoretically and from the point of view of meadow and pasture management, in order to ensure the feed basis which is indispensable in cattle farming (ANDREJEV 1972, 1975, DENT—ANDERSON 1971, KOVÁCS—CINKÓCZKY 1974, RABOTNOV 1974, SZABÓ, 1977, Soó 1968—1973). The development of single-species grass stands which steadily ensure large yields, the extent of weed growth in them, and the invasion and stabilization of other species characteristic of the region do not only raise theoretical questions of phytocenology, since the relatively rapid process of succession taking place in grasses, as well as the changes occurring year by year under the influence of environmental factors and as a result of irrigation and fertilization, have a considerable effect both on the quantity and quality of yield, and on the value of components utilizable by the animals.

One of the objects of our present experiments, which form part of our grass production studies, was to find out how a grass stand of relatively constant floristic and population composition evolves under identical conditions of water and nutrient supply in a meliorated solonetz meadow soil, and how the existing ecological conditions and the cultural practices employed influence the composition, change and succession of species and population in grasses planted with one species during the years following the plantation.

The experiments were carried out in the Káka district of the Szarvas State Farm, in the K-10 block of the Plesovszki pasture during the vegetation periods of 1973—1976. The grass experiment was laid out under identical agrotechnical conditions (nutrient level, water

supply, spacing) with six perennial species: *Festuca arundinacea*, *Festuca pratensis*, *Bromus inermis*, *Dactylis glomerata*, *Trifolium repens* f. *giganteum* and *Lotus corniculatus*.

The 100 m^2 trial plots were laid out in a random block design with four replications. After previous autumn and spring preparation of the soil and the application of 4500 kg/ha $CaCO_3$ in autumn and 45 kg/ha nitrogen active agent in spring, the six species mentioned were sown on 13th March 1973 with a row space of 12 cm. The fertilization of the grasses at a rate of 68 kg/ha, N, 18 ka/ha P_2O_5 and 40 kg/ha K_2O was carried out every year, usually after the fourth growth had been cut. The major characteristics of the solonetz meadow soil are summarized in Table 1 and the meteorological data of the area in Table 2. The 300—400 mm irrigation water applied with a sprinkler during the vegetation period resulted in a grass stand of mesophylous character with a medium water requirement. In the neighbourhood of Szarvas poor and furrowed fescue grass-lands prevail.

Table 1

Characteristic soil data

Depth of sample taking, cm	pH		Humus, %	Total N, %	P_2O	K_2O	$CaCO_3$, %	Viscosity index	Total salt, %	Capillary water lift	
	H_2O	KCl			mg/100 g soil					5 hr	24 hr
0—15	7.10	6.80	3.29	0.17	89.00	86.73	0.42	50	0.20	90	160
15—30	7.40	7.05	2.48	0.14	43.00	32.52	0	50	0.15	70	175
30—45	7.55	7.05	3.69	0.09	16.42	21.68	0	59	0.15	50	135
45—60	7.95	7.20	1.41	0.07	11.20	19.27	0.84	61	0.17	30	115
60—90	7.50	7.05	0.94	0.04	7.05	19.27	2.95	59	0.40	80	225

The phytocenological surveys of the grass stands on trial plots were made in the four vegetation periods before cutting the growth. In this way 68 cenological surveys were made per stand, using the Braun–Blanquet method and scale; they are summed up according to an economic categorization of the species in Tables 3—8.

1. Grass stand planted with *Festuca arundinacea* Schreb. (Table 3). From 350 g of seed sown over an area of 100 m^2 102,500 seedlings developed. In the sowing year besides the species sown a relatively low number (12—14) of mainly annual weed species (of the *Secalinetalia* ass. series) were found, amounting to 12.83% by weight of the total yield. In 1974 the species number doubled (26—29), due mainly to the invasion of perennial species, but their percentage by weight fell to an average of 3.46%. In 1975 the species sown gave an 80—85% cover while the invader perennial grasses, papilionaceae and annual weeds covered 10—15% of the surface. The weight percentage of the invader species rose to 7.45% and the number of species ranged between 31 and 33. In 1976, when the grass closed up, a relatively steady floristic stand developed with 24—27 mostly perennial species. Their weight percentage in the total yield was 9.16% in dry matter. When considering the number of species in the successive growths it is found that the number of invader species was the highest in the first (spring) growth, decreasing by 2—7 species by the autumn. The same applies to the weight percentage of the invader species, which was 4—5 times lower in autumn than in spring.

Table 2

Characteristic meteorological data (Szarvas and its environs, 1973—1976)

Temperatures

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
50 year aver.	—2	—0.1	5.2	10.5	15.9	19.3	21.5	20.5	16.5	10.5	4.5	0.2
1973 mean °C	—1.1	—1.2	3.8	9.6	16.2	20.5	20.5	21.6	16.9	9.3	—2.3	—0.7
1974 mean °C	0.9	5.3	8.9	9.5	15.7	11.3	19.4	22.9	18.3	8.7	2.9	5.7
1975 mean °C	0.8	—0.5	7.6	10.1	17.5	18.9	19.7	22.1	19.7	7.1	4.3	—1.9
1976 mean °C	—0.4	—2.1	2.2	11.8	15.9	18.7	28.3	18.3	15.6	12.0	6.9	3.4

Natural and artificial water supply (mm)

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Total	III—IX
50-year average	27	29	31	42	58	59	54	48	40	46	51	39	524	332
1973														
precip.	11.5	26.3	5.0	54	38.8	132.5	49.4	33.8	10.2	27.7	5.1	24.7	418.8	323.5
irrig.	—	—	40	40	120	—	60	60	40	—	—	—	400	400
1974														
precip.	6.5	21.2	7.4	33.3	76.5	121	47	105.4	24.7	112.8	23.6	31.8	611.2	415.3
irrig.	—	—	—	80	60	40	60	—	60	—	—	—	300	300
1975														
precip.	6.5	3.1	25.7	19.2	85.1	133.2	100	68.7	80.2	15.3	7	16.3	560.3	512.1
irrig.	—	—	—	80	60	—	80	80	—	—	—	—	300	300
1976														
precip.	29.8	1.5	37.8	25.6	56	33.3	17.3	13.8	68.2	31.8	18.8	70.4	404.3	252
irrig.	—	—	—	40	40	80	120	120	—	—	—	—	400	400

Monthly averages of relative humidity (%)

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1973	89	77	74	75	67	71	68	64	69	77	70	81
1974	86	75	66	62	73	72	71	76	78	85	82	86
1975	88	77	75	74	73	73	86	93	89	72	75	81
1976	75	72	67	54	58	56	50	59	75	78	81	83

Table 3

Changes in the sown grass

Date of survey			1973				
			3. V.	5. VI.	5. VII.	28. VIII.	3. X.
Growth			1st	2nd	3rd	4th	5st
General cover, %			70	70	80	80	80
H	Eua	*Festuca arundinacea*	3—4	3—4	4	4	4
H	Eua	*Festuca pratensis*	—	—	—	—	—
H	Eu	*Lolium perenne*	—	—	—	—	—
H	Eua	*Dactylis glomerata*	—	—	—	—	—
Th-TH	Eua	*Bromus arvensis*	—	—	—	—	—
Th-TH	M	*Lolium multiflorum*	—	—	—	—	—
Th	Cos	*Echinochloa crus-galli*	—	—	—	—	—
H	Eua	*Trifolium repens gigant.*	—	—	—	—	—
H	Eua	*Trifolium repens*	—	—	—	—	—
H	Eua	*Lotus corniculatus*	—	—	—	—	—
H	Eua	*Trifolium pratense*	—	—	—	—	—
H	Eua	*Lathyrus tuberosus*	+	—	—	—	—
Th	Eua	*Lamium amplexicaule*	1—2	1	1	1	1
G	Eua	*Cirsium arvense*	+	+	+	+	+
H	Cos	*Taraxacum officinale*	+	+	+	+	+
Th	Cpl	*Polygonum convolvulus*	+	+	+	+	+
Th	Cos	*Stellaria media*	1	+	+	+	+
Th	Cos	*Anagallis arvensis*	+	+	+	+	+
Th	Cos	*Capsella bursa-pastoris*	+	+	+	+	+
Th	Cos	*Geranium pusillum*	+	—	—	—	—
H	Eua	*Stellaria graminea*	—	—	+	+	+
Th	Eu	*Adonis aestivalis*	—	—	—	—	—
H	Eua	*Rumex crispus*	—	—	—	—	—
Th-TH	Eua	*Matricaria inodora*	+	+	+	+	+
Th	Eua	*Galium aparine*	—	—	—	—	—
Th-TH	Cos	*Cerastium caespitosum*	—	—	+	+	+
Th	Cos	*Chenopodium album*	+	—	—	—	—
H-G	Cos	*Convolvulus arvensis*	—	—	—	—	—
Th	M	*Crepis setosa*	—	—	—	—	—
H	Eu	*Ranunculus sardous*	—	—	—	—	+
Th	Cos	*Centaurea cyanus*	—	—	—	—	—
Th	Eua	*Lepidum draba*	—	—	—	—	—
Th	M	*Stachys annua*	—	—	—	—	—
Th	Cos	*Hibiscus trionum*	—	—	—	—	—
Th	M	*Papaver dubium*	—	—	+	+	+
Th	Eua	*Lithospermum arvense*	—	—	—	—	—
H	Cos	*Sonchus arvensis*	—	—	—	—	—
Th	Eua	*Thlaspi arvense*	+	—	—	—	—
Th	Eua	*Malva neglecta*	—	—	—	—	—
H	Eua	*Plantago major*	—	—	—	—	—
H	Eua	*Ranunculus repens*	—	—	—	—	—
Th	Cos	*Polygonum aviculare*	—	—	—	—	—
Th	Adv	*Amaranthus albus*	—	—	—	—	—
Th	Cos	*Amaranthus retroflexus*	—	—	—	—	—
Th	Cos	*Chenopodium hybridum*	—	—	—	—	—
TH	Eua	*Daucus carota*	—	—	—	—	—
Th	Eua	*Descurainia sophia*	—	—	—	—	—
Th	Eua	*Veronica hederifolia*	+	+	—	—	—

stand of Festuca arundinacea

1974				1975				1976			
3. V.	20. VI.	8. VII.	28. IX.	21. V.	20. VI.	15. VII.	25. IX.	11. V.	15. VI.	24. VIII.	23. IX.
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
80	90	100	1000	90	90	95	95	90	95	100	100
4	4	4	4–5	5	4	4	4	5	5	5	5
+	+	+	1	1	1–2	1–2	1–2	1	1	1	1
+	+	1	1	1	1	+–2	+–2	+–1	+–1	+–1	1
–	–	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	–	–	–	–
–	–	–	–	–	+	+	+	+	+	+	+
–	–	–	–	–	+	+	+	+–1	+–1	+–1	1
+	+	+	+	1	+–3	+–3	+–3	1	+	+	+
–	–	+	+	+	+	+	+	+	+	+	+
–	–	+	+	+	+	+	+	+	+	+	+
–	–	–	–	–	+	+	+	+	+	+	+
+	–	–	–	+	–	–	–	+	–	–	–
1–2	1	1	1	1–2	1	1	1	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	1	+
+	+	+	+	+	+	+	+	+	+	+	+
+–1	+	+	+	1	+	+	+	–	–	+	+
+	+	+	+	+	+	+	+	–	–	–	–
+	+	+	+	+	+	+	+	–	–	–	–
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+–2	1	+	+	+	+	–	–	–	–
+	+	+	+	+	+	+	+	+	+	–	–
–	–	+	+	+	+	+	+	+	+	–	+
1–2	+	–	–	+	+	–	–	+	–	–	–
+	+	+	+	+	+	+	+	–	–	–	–
+	+	+	–	+	–	–	–	–	–	–	–
+	+	+	–	+	+	–	–	–	+	+	–
–	–	–	–	–	+	+	+	+	+	+	+
–	–	–	–	–	+	+	+	+	+	+	+
+	–	–	–	+	–	–	–	+	+	+	+
–	–	+	+	+	+	+	+	–	–	–	–
+	+	–	–	+	+	+	+	–	–	–	–
–	–	+	+	+	+	+	+	–	–	–	–
–	–	–	–	–	–	+	+	+	+	+	+
+	+	+	–	–	–	–	–	–	–	–	–
1	+	–	–	+	+	–	–	–	–	–	–
–	+	+	+	+	–	–	–	–	–	–	–
+	+	–	–	+	–	–	–	–	–	–	–
–	+	+	+	+	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	+	+	+	+
–	–	–	–	–	–	–	–	+	+	+	+
–	–	–	–	–	–	–	–	+	+	+	+
–	–	–	–	–	+	+	+	–	–	–	–
–	–	+	+	–	–	–	–	–	–	–	–
–	–	+	+	–	–	–	–	–	–	–	–
–	–	–	–	–	–	+	+	–	–	–	–
+	+	–	–	–	–	–	–	–	–	–	–
+	+	–	–	+	+	–	–	+	–	–	–

Table 4

Changes in the sown grass stand

Date of survey			1973				
			3. V.	5. VI.	5. VII.	28. VIII.	3. X.
Growth			1st	2nd	3rd	4th	5th
General cover, %			90	90	90	95	100
H	Eua	*Festuca pratensis*	4	4	4	4	5
Th	Cos	*Echinochloa crus-galli*	+	+	+	+	+
H	Eua	*Dactylis glomerata*	—	—	—	—	—
H	Eua	*Festuca arundinacea*	—	—	—	—	—
H	Eu	*Lolium perenne*	—	—	—	—	—
Th-TH	Eua	*Bromus arvensis*	—	—	—	—	—
H	K	*Bromus inermis*	—	—	—	—	—
Th	Eua	*Bromus mollis*	—	—	—	—	—
Th-TH	M	*Lolium multiflorum*	—	—	—	—	—
G	Eua	*Agropyron repens*	—	—	—	—	—
H	Cpl	*Poa pratensis*	—	—	—	—	—
H	Eua	*Trifolium repens gigant.*	—	—	—	—	—
H	Eua	*Trifolium repens*	—	—	—	—	—
Th-TH	Eua	*Melilotus officinalis*	—	—	—	—	—
H	Eua	*Lathyrus tuberosus*	—	—	+	+	+
H	Eua	*Lotus corniculatus*	—	—	—	—	—
H	Eua	*Trifolium fragiferum*	—	—	—	—	—
H	Adv	*Medicago sativa*	—	—	—	—	—
H	Eua	*Vicia sepium*	—	—	—	—	—
Th	Cos	*Anagallis arvensis*	+	+	+	+	+
G	Eua	*Cirsium arvense*	+	+	+	+	+
H-G	Cos	*Convolvulus arvensis*	+	+	+	+	+
Th	Adv	*Erigeron canadensis*	+	+	+	+	+
Th	Cos	*Hibiscus trionum*	+	+	+	+	+
Th	Cos	*Geranium pusillum*	+	+	+	+	+
Th	Eua	*Lamium amplexicaule*	+	+	+	+	+
Th	Cpl	*Polygonum convolvulus*	+	+	+	+	+
H	Eu	*Ranunculus sardous*	+	+	+	+	+
H	Eua	*Rumex crispus*	+	+	+	+	+
Th	Cos	*Stellaria media .*	+	+	+	+	1
H	Eua	*Stellaria graminea*	+	+	+	+	+
H	Cos	*Taraxacum officinale*	+	+	+	+	+
Th	Eu	*Adonis aestivalis*	3+	+	+	+	+
Th-TH	Cos	*Cerastium caespitosum*	—	—	+	+	+
Th	Eua	*Galium aparine*	+	+	+	+	+
Th	Cos	*Capsella bursa-pastoris*	—	—	—	+	+
Th	Cos	*Sonchus asper*	—	—	—	—	+
Th	Cos	*Polygonum aviculare*	—	—	—	—	—
Th-TH	Eua	*Matricaria inodora*	—	—	—	—	—
H	Cos	*Sonchus arvensis*	—	—	—	—	—
Th	M	*Papaver dubium*	—	—	—	+	+
Th	Cos	*Chenopodium album*	+	+	—	—	—
Th	Cos	*Chenopodium hybridum*	+	+	—	—	—
Th	M	*Crepis setosa*	—	—	—	—	—
Th	Eua	*Camelina microcarpa*	+	+	—	—	—
Th	Adv	*Amaranthus albus*	+	—	—	—	—
Th	Eua	*Veronica hederifolia*	—	—	—	—	—
Th	Cos	*Amaranthus retroflexus*	—	—	—	—	—
Th	Eua	*Malva neglecta*	—	—	—	—	—
Th	Eua	*Descurainia sophia*	—	—	—	—	—
Th	M	*Bifora radians*	—	—	—	—	—
Th	Eua	*Papaver rhoeas*	—	—	—	—	—
H	Eua	*Rorippa silvestris*	—	—	—	—	—
Th	Eua	*Thlaspi arvense*	—	—	—	—	—
H	Eua	*Centaurea scabiosa*	—	—	—	—	—

of Festuca pratensis 1973—1976

1974				1975				1976			
3. V.	20. VI.	8. VII.	28. IX.	21. V.	20. VI.	15. VII.	25. IX.	11. V.	15. VI.	24. VIII.	23. IX.
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
100	100	100	100	70	85	90	90	90	85	80	80
5	5	5	5	3—4	3	3	2—3	2	1—2	1—2	1
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	1	1	1—2	1—2	1—2	1—2	1—2	1—2
+	+	+	+	+	+	+	+	+	+	+—1	+—1
+	+	+	+	+	+	1	1	1	+	+	+
+	+	+	+	+	+	+	+	—	—	—	—
—	—	—	—	+	+	+—1	+—1	+—1	+—1	+—1	+—1
—	—	—	—	+	+	+	+	—	—	—	—
—	—	—	—	+	+	+	+	—	—	—	—
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
+	+	+	+	+	1	1—2	1—2	1—2	1—2	1—2	1—2
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	—	—	—	—
—	—	+	+	+	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
+	+	—	—	—	—	—	—	—	—	—	—
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+—1	+—1	+—1	+
+	+	+	+	+	+	+	+	1	1—2	1—2	+—1
+	+	+	+	+	+	+	+	+	+	+—1	1
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	1—2	1—2	1—2	1—2
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+—1	1	+	+	1	+	1	1	1	1
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+—1	1	2—3	2—3	2—3	3—4
—	+	+	+	+	+	+	+	+	+	+	+
+—2	1	1	1	1	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	—	—	—
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	1	1	1
+—1	1	+	+	+	+	+	+	+	+	—	—
—	—	—	—	+	+	+	+	+	+	+	+
+	+	+	+	+	+	—	—	—	—	—	—
+	+	+	—	+	+	—	—	—	—	—	—
+	+	+	—	+	+	—	—	—	—	—	—
—	—	—	—	—	+	+	+	+—1	+—1	+—1	+
+	+	—	—	+	+	—	—	—	—	—	—
+	—	—	—	+	+	—	—	+	+	—	—
+	+	+	+	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
+	+	+	—	—	—	—	—	—	—	—	—
—	—	+	+	—	—	—	—	—	—	—	—
—	—	+	+	—	—	—	—	—	—	—	—
+	+	—	—	—	—	—	—	—	—	—	—
+	+	—	—	—	—	—	—	—	—	—	—
+	+	+	+	—	—	—	—	—	—	—	—

Table 5
Changes in the sown grass stand

		Date of survey	1973				
			3. V.	5. VI.	5. VII.	28. VIII.	3. X.
		Growth	1st	2nd	3rd	4th	5th
		General cover, %	70	75	80	80	80
H	K	*Bromus inermis*	3—4	3	3	3—4	3—4
H	Eua	*Dactylis glomerata*	—	—	—	—	—
H	Eua	*Festuca pratensis*	—	—	—	—	—
H	Eu	*Lolium perenne*	—	—	—	—	—
Th	Eua	*Bromus sterilis*	—	—	—	—	—
Th-TH	Eua	*Bromus arvensis*	—	—	—	—	—
Th	Eua	*Setaria viridis*	—	—	—	—	+
Th-TH	M	*Lolium multiflorum*	—	—	—	—	—
Th	Cos	*Echinochloa crus-galli*	—	—	—	—	—
G	Eua	*Agropyron repens*	—	—	—	—	—
H	Eua	*Festuca arundinacea*	—	—	—	—	—
H	Eua	*Trifolium repens gigant.*	+	+	+	+	+
Th TH	Eua	*Melilotus officinalis*	+	+	+	+	+
H	Eua	*Vicia sepium*	+	+	+	+	+
H	Eua	*Lathyrus tuberosus*	—	—	+	+	+
H	Eua	*Lotus corniculatus*	—	—	—	—	—
H	Eua	*Trifolium repens*	—	—	—	—	—
H	Eua	*Trifolium pratense*	—	—	—	—	—
Th	Cos	*Stellaria media*	1—2	2	2	1—2	+—2
Th	Eua	*Lamium amplexicaule*	1—2	1	1	2	2
Th-TH	Eua	*Matricaria inodora*	+	+	+	+	+
Th	Cos	*Capsella bursa-pastoris*	+	+	+	+	+
G	Eua	*Cirsium arvense*	+	+	+	+	+
H	Eua	*Rumex crispus*	+	+	+	+	+
H	Cos	*Taraxacum officinale*	+—1	+	+	+	+
Th	Cos	*Hibiscus trionum*	+	+	+	+	+
Th-TH	Cos	*Cerastium caespitosum*	+	+	+	+	1
Th	Eua	*Galium aparine*	+	+	+	+	+
H	Eua	*Ranunculus sardous*	+	+	+	+	+
Th	Cos	*Viola arvensis*	+	+	+	+	+
Th	Cos	*Geranium pusillum*	+	+	+	+	+
Th	Cos	*Sonchus asper*	—	—	+	+	+
Th	Eu	*Adonis aestivalis*	+	—	—	+	+
Th	Cos	*Anagallis arvensis*	—	—	—	—	—
Th	Adv	*Amaranthus albus*	—	—	—	—	—
Th	Cos	*Amaranthus retroflexus*	+	+	+	+	+
Th	Eua	*Descurainia sophia*	+	+	+	+	+
Th	Cos	*Centaurea cyanus*	—	—	—	—	—
Th	M	*Consolida orientalis*	—	—	—	—	—
Th	Cpl	*Polygonum convolvulus*	—	—	—	—	—
Th	Cos	*Polygonum aviculare*	—	—	—	—	—
Th	M	*Stachys annua*	—	—	—	—	—
Th	Eua	*Matricaria chamomilla*	—	—	—	—	—
Th	M	*Papaver dubium*	—	—	—	—	—
Th	Eua	*Camelina microcarpa*	—	—	—	+	+
H	Eua	*Stellaria graminea*	—	—	—	—	—
H	Eua	*Cichorium intybus*	—	—	—	—	—
H	G	*Convolvulus arvensis*	—	—	—	—	—
Th	M	*Crepis setosa*	—	—	—	—	—
H	Eua	*Plantago major*	—	—	—	—	—
H	Eua	*Rorippa silvestris*	—	—	+	+	+
Th	Eua	*Veronica hederifolia*	—	—	+	+	+
Th	Eua	*Thlaspi arvense*	—	—	+	+	+
Th	Eua	*Lepidium draba*	—	—	—	+	+
Th	Cos	*Chenopodium botrys*	—	—	—	—	—
Th	Cos	*Chenopodium album*	—	—	—	—	—
Th	Adv	*Erigeron canadensis*	—	—	—	—	—
H	M	*Mentha pulegium*	—	—	—	—	—

of Bromus inermis 1973—1976

1974				1975				1976			
3. V.	20. VI.	8. VII.	28. IX.	21. V.	20. VI.	15. VII.	25. IX.	11. V.	15. VI.	VIII.24.	23. IX.
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
80	80	80	90	95	90	90	90	80	80	80	80
4	4	4	4	5	4	4	4	3—4	3—4	3	3
+	+	+	+	+—1	1—2	1—2	1—2	1	1	1	1
+	1	1	1	1	1	1	1	1	1	1	1
+	+	+	1	1	1	1	1	1	1	1	1
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	—	—	—	—
—	—	+	—	+	+	+	+	—	—	—	—
—	—	—	—	—	—	—	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
+	+	+	1—2	1—2	2	2	2	1—2	1—2	1—2	1—2
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	—	—	+	+	—	—	—	—
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+	+	+
2	1	1—2	1—2	1—2	1—2	1	1	1	1	1	1
1—2	1	1	1	1	1	1	1	+	+	+	+
+—1	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	1	1	1	1
+	+	+	+	+	+	+	+	+	+	+	+
1—2	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	—	—	—	—
+	—	—	—	+	+	+	—	—	+	+	+
+	+	+	+	+	+	—	—	—	+	+	—
+	+	+	+	+	+	—	—	—	—	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	—	—	—	—	—	—	+	+	+
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	+	+—1	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	—	—	—	—
—	+	+	+	+	+	+	+	—	—	—	—
—	—	+	+	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+—1	+—1	1	1
—	—	—	—	+	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+	+	+
+	—	—	—	+	+	—	—	—	—	—	—
+	—	—	—	+	+	—	—	—	—	—	—
+	—	—	—	—	—	—	—	—	—	—	—
+	—	—	—	—	—	—	—	—	—	+	—
+	+	+	+	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+

Table 6

Changes in the sown grass stand

Date of survey			1973				
			3. V.	5. VI.	5. VII.	28. VIII.	3. X.
Growth			1st	2nd	3rd	4th	5th
General cover, %			85	90	90	80	85
H	Eua	*Dactylis glomerata*	5	5	5	5	5
Th	Cos	*Echinochloa crus-galli*	+	+−1	+−1	1	+−1
H	K	*Bromus inermis*	−	−	−	−	−
H	Eua	*Festuca arundinacea*	−	−	−	−	−
H	Eu	*Lolium perenne*	−	−	−	−	−
Th	Eua	*Setaria viridis*	+	+	+	+	1
H	Cpl	*Poa angustifolia*	−	−	−	−	−
Th	Eua	*Bromus mollis*	−	−	−	−	−
H	Eua	*Trifolium repens gigant.*	+	+	+	+	+
H	Eua	*Lotus corniculatus*	−	−	−	−	−
G	Eua	*Cirsium arvense*	+	+	+	+	+
Th	Eua	*Lamium amplexicaule*	+	+	+	+	1
Th	Cos	*Amaranthus retroflexus*	+	+	+	+	+
Th	Cos	*Anagallis arvensis*	+	+	+	+	+
H-G	Cos	*Convolvulus arvensis*	+	−	−	+	−
H	Cos	*Taraxacum officinale*	+	+	+	+	+
TH	Eua	*Daucus carota*	+	+	+	+	+
Th	Adv	*Erigeron canadensis*	−	−	−	−	−
Th	Cos	*Capsella bursa-pastoris*	−	−	−	+	+
Th	Cos	*Hibiscus trionum*	+	+	+	+	+
Th	Cos	*Polygonum aviculare*	−	−	−	−	−
H	Eua	*Cichorium intybus*	+	+	+	+	+
H	Eua	*Stellaria graminea*	−	−	−	+	+
Th	Cos	*Chenopodium album*	1	1	+	+	−
Th	Eua	*Matricaria chamomilla*	−	+	−	−	+
Th-TH	Eua	*Matricaria inodora*	−	−	−	−	+
H	Cos	*Sonchus arvensis*	−	−	−	−	−
TH	Eua	*Conium maculatum*	+	+	+	+	+
Th	Eua	*Malva neglecta*	+	+	+	+	+
Th	Cos	*Geranium pusillum*	−	−	−	−	−
Th	M	*Crepis setosa*	−	−	−	−	−
Th	Cpl	*Polygonum convolvulus*	−	−	−	−	−
H	Eua	*Plantago major*	−	−	−	−	−
Th	Cos	*Portulacea cleracea*	−	−	−	−	−
Th	Eua	*Thlaspi arvense*	−	−	−	−	−
Th	Eua	*Adonis aestivalis*	−	−	−	−	−
Th	Cos	*Sonchus asper*	−	−	−	−	−

In four years a total of 48 species was found in the trial plots. A comparison between the grass stands of the first and fourth years on the basis of the ecological and area spectra of the species gives the same result.

Ecological spectrum:

1973: Th-58.82%, H-23.53%, Th-TH-11.76%, G-5.89%
1976: Th-36.67%. H-46.67%, Th-TH-10%, G-3.33%, H-G-3.33%
Area spectrum:
1973: Eua-47.06%. Cos-41.18%, Cpl-5.88%, M-5.88%
1976: Eua-53.34%, Cos-26.67%, Eu-10%, M-6.66%, Cpl-3.33%

of Dactylis glomerata 1973—1976

1974				1975				1976			
3. V.	20. VI.	8. VII.	28. IX.	21. V.	20. VI.	15. VII.	25. IX.	11. V.	15. VI.	24. VIII.	23. IX.
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
90	85	80	80	85	80	80	75	75	80	80	80
5	4—5	4—5	4—5	4	4	4	4	3—4	3—4	3—4	3—4
—	+	+	+	+	+	+	+	1	1	1	1
—	+	+	+	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	1	1	1
—	—	—	+	+	+	+	+	+	1	1	1
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	—
+	+	+	+	1	1	1	1	+	1	1	1
—	—	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	1	1	+
—	+	+	—	+	+	+	+	+	+	+	+
+	+	+	+	+	+	—	—	+	+	+	+
—	+	+	—	+	+	+	+	+	+	+	+
+	+	+	+	1	+	1	1	1—2	1—2	1—2	1
—	+	+	+	+	+	+	+	—	—	—	—
—	+	+	+	+	+	+	+	+	+	+	+
+	+	—	+	+	+	+	—	—	—	+	+
—	—	—	—	—	—	+	—	+	+	+	+
—	—	—	+	+	+	+	+	+	+	+	+
—	+	—	—	—	—	+	+	—	—	—	—
+	+	+	—	—	+	+	+	—	—	—	—
—	—	—	—	—	—	—	—	+	+	+	+
—	+	—	—	+	+	+	—	—	—	—	—
+	+	+	+	—	—	—	—	—	—	+	+
+	+	+	—	—	—	—	—	+	+	+	+
+	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
+	+	+	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	+	+	+	—
—	—	—	—	—	—	—	—	+	+	+	—

Thus, beside the species sown many other grass and papilinaceous species whose feed value was more or less identical with that of the species sown, became established in the stand within a relatively short time. Of the weeds, those with rhizomes or stolons, and annual weeds which produce seed before mowing primarily survived. The green crop from the grass was 626.9 q/ha (164.65 q)/ha in dry matter) on the average of four years.

2. Grass stand sown with *Festuca pratensis* Huds. From 500 g seed sown over an area of 100 m² an average of 85,000 seedlings came up. In the sowing year the cover of mainly annual weeds was 10—12% and their weight ratio in the total yield 6.6%. The number of species occurring in the trial plots was 19—22. In 1974 *Festuca pratensis* already formed a closed stand with a 90—95% cover, but, due mainly to the invasion of perennial species, the number of

Table 7

Changes in the sown grass stand of

Date of survey			1973				
			3. V.	5. VI.	5. VII.	28. VIII.	3. X.
Growth			1st	2nd	3rd	4th	5th
General cover, %			70	75	75	80	80
H	Eua	*Trifolium repens gigant.*	3—4	3—4	3—4	4	4
H	Eua	*Trifolium repens*	+	+	+	+	+
H	Adv	*Medicago sativa*	—	—	—	—	—
H	Eua	*Trifolium pratense*	—	—	—	—	—
Th-TH	Eua	*Melilotus officinalis*	—	—	—	—	—
H	Eua	*Lathyrus tuberosus*	—	—	—	—	—
H	Eua	*Dactylis glomerata*	+	+	+	+	+
H	Eua	*Festuca pratensis*	—	—	+	+	+
H	Eu	*Lolium perenne*	—	—	—	—	—
Th	Cos	*Echinochloa crus-galli*	—	—	—	—	—
Th-TH	M	*Lolium multiflorum*	—	—	—	—	—
Th	Eua	*Setaria viridis*	—	—	—	—	+
H	Eua	*Festuca arundinacea*	—	—	—	—	—
h	Eua	*Hordeum murinum*	—	—	—	—	—
Th-TH	Eua	*Bromus arvensis*	—	—	—	—	—
Th	Cos	*Stellaria media*	1—2	1—2	1	1—2	1—2
H	Eua	*Cichorium intybus*	+	+	+	+	+
G	Eua	*Cirsium arvense*	+	+	+	+	+
Th	Cos	*Anagallis arvensis*	+	+	+	+	+
H-G	Cos	*Convolvulus arvensis*	+	+	+	+	+
Th	Cos	*Geranium pusillum*	+	+	+	+	+
H	Cos	*Taraxacum officinale*	+	+	+	+	+
Th	Eua	*Lamium amplexicaule*	1—2	1—2	+—1	+—1	+
Th	Cpl	*Polygonum convolvulus*	+	+	+	+	+
Th-TH	Cos	*Cerastium caespitosum*	+	+	+	+	+
Th	Eua	*Galium aparine*	+	+	+	+	+
Th	Eua	*Malva neglecta*	—	—	+	+	—
Th	Cos	*Sonchus asper*	—	—	+	—	—
H	Eua	*Centaurea jacea*	—	—	—	—	—
Th	Eua	*Camelina microcarpa*	+	—	—	+	—
Th	Eua	*Matricaria chamomilla*	—	—	—	—	—
Th	Cos	*Polygonum aviculare*	+	—	—	+	—
Th	Cos	*Hibiscus trionum*	+	+	—	—	—
Th-TH	Eua	*Matricaria inodora*	—	—	—	+	+
Th	Cos	*Centaurea cyanus*	—	—	—	—	—
H	Eua	*Ranunculus sardous*	—	—	—	—	—
Th	Adv	*Erigeron canadensis*	—	—	—	—	+
Th	Cos	*Viola arvensis*	+	—	—	—	—
H	Eua	*Centaurea scabiosa*	—	—	—	—	—
Th	Eua	*Lamium purpureum*	+	—	—	—	—
H	Eua	*Rumex crispus*	—	—	—	—	+
Th	Eua	*Lepidium draba*	—	—	—	—	—
Th	Eua	*Lithospermum arvense*	+	—	—	—	—
H	Eua	*Rorippa silvestris*	—	—	—	—	—
Th	Eu	*Adonis aestivalis*	+	—	—	—	—
H	Eua	*Stellaria graminea*	—	—	—	—	+
Th	Eua	*Descurainia sophia*	+	—	—	—	—
Th	Cos	*Sinapis arvensis*	+	—	—	—	—
Th	M	*Papaver dubium*	+	—	—	—	—
Th	M	*Stachys annua*	—	—	—	—	—
Th	Cos	*Chenopodium album*	—	—	—	—	—

Trifolium repens f. giganteum 1973—1976

1974				1975				1976			
3. V.	20. VI.	8. VII.	28. IX.	21. V.	20. VI.	15. VII.	25. IX.	11. V.	15. VI.	24.VIII.	23. IX.
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
80	80	80	85	90	100	100	100	100	100	100	100
4—5	4	4	4	4	5	5	5	5	5	5	5
+	+—1	+	+	+	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+	+	+
—	—	—	—	—	—	+	+	+	+	+	+
+	+	+	—	—	—	—	—	—	—	—	—
+	+	—	—	—	—	—	—	—	—	—	—
+	+	+	+	+	+	+	+	+	+	+	+
+	+	1	+	+	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+	+	+
—	—	—	—	—	+	+	+	+	+	+	+
—	—	—	+	—	—	+	+	—	—	—	+
—	—	—	—	—	—	—	—	+	+	+	+
—	—	—	—	—	—	—	—	+	+	+	+
—	—	+	+	—	—	—	—	—	—	—	—
+	+—1	+—1	1—2	+—1	+—1	+	+—1	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
1—3	+—1	+—1	+—1	1—2	1—2	1	+—1	+	—	—	—
+	+	+	+	+	+	+	+	+	—	—	—
+	+	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	+	—	—	—	—
—	—	+	+	+	+	+	+	—	—	—	—
+	+	+	+	+	+	+	—	—	—	—	—
—	+	+	+	+	+	+	+	—	—	—	—
+	+	—	+	+	—	—	—	—	—	—	—
—	—	+	+	+	+	+	+	—	—	—	—
—	—	+	+	+	+	—	—	—	—	—	—
—	—	—	—	—	—	+	+	+	+	—	—
+	—	—	—	—	—	—	—	—	—	+	+
—	+	+	+	+	—	—	—	—	—	—	—
+	+	+	+	—	—	—	—	—	—	—	—
—	—	—	—	—	—	+	+	—	—	—	+
+	+	+	—	—	—	—	—	—	—	—	—
+	+	+	+	—	—	—	—	—	—	—	—
+	+	+	—	—	—	—	—	—	—	—	—
—	—	+	+	+	—	—	—	—	—	—	—
+	+	+	—	—	—	—	—	—	—	—	—
+	—	—	—	+	—	—	—	—	—	—	—
+	+	+	—	—	—	—	—	—	—	—	—
+	—	—	—	+	—	—	—	—	—	—	—
—	—	—	—	+	+	—	—	—	—	—	—
+	—	—	—	+	—	—	—	—	—	—	—
+	—	—	—	+	—	—	—	—	—	—	—
—	—	+	+	—	—	—	—	—	—	—	—
—	—	+	+	—	—	—	—	—	—	—	—

Table 8

Changes in the sown grass stand

		Date of survey	1973				
			3. V.	5. VI.	5. VII.	28. VIII.	3. X.
		Growth	1st	2nd	3rd	4th	5th
		General cover, %	100	100	90	90	90
H	Eua	*Lotus corniculatus*	1—2	1—2	1—2	1—2	2
H	Adv	*Medicago sativa*	—	—	—	+	+
H	Eua	*Trifolium repens*	—	—	—	—	+
H	Eua	*Trifolium pratense*	—	—	—	—	—
H	Eua	*Trifolium repens gigant.*	—	—	—	—	—
Th	Eua	*Vicia tetrasperma*	+	+	+	—	—
H	Eua	*Vicia sepium*	+	+	+	+	+
H	Eua	*Lathyrus tuberosus*	+	+	+	+	+
Th	Cos	*Echinochloa crus-galli*	—	—	—	+	+
H	K	*Bromus inermis*	—	—	—	—	—
H	Eua	*Dactylis glomerata*	—	—	—	—	—
H	Eua	*Festuca pratensis*	—	—	—	—	—
H	Eua	*Festuca arundinacea*	—	—	—	—	—
H	Eu	*Lolium perenne*	—	—	—	—	—
Th	Eua	*Bromus mollis*	—	—	—	—	—
Th-TH	M	*Lolium multiflorum*	—	—	—	—	—
Th-TH	Eua	*Bromus arvensis*	—	—	—	—	—
Th	Eua	*Setaria viridis*	—	—	—	—	—
H	Cpl	*Poa pratensis*	—	—	—	—	—
HH	Cpl	*Typhoides arundinacea*	—	—	—	—	—
G	Eua	*Agropyron repens*	—	—	—	—	—
Th	Eua	*Lamium amplexicaule*	2—3	2—3	2—3	1—3	1—3
Th	Cos	*Stellaria media*	+—3	+—2	+—2	+—2	+—1
H	Cos	*Taraxacum officinale*	+	+	+	+	+
Th	Cos	*Geranium pusillum*	+	+	+	+	+
Th	Cos	*Capsella bursa-pastoris*	+	+	+	+	+
Th	Cos	*Anagallis arvensis*	+	+	+	+	+
G	Cos	*Cirsium arvense*	+	+	+	+	+
H	Eua	*Rumex crispus*	+	+	+	+	+
H-G	Cos	*Convolvulus arvensis*	+	+	+	+	+
Th-TH	Cos	*Cerastium caespitosum*	+—1	+	+	+	+
Th	Cos	*Polygonum aviculare*	—	—	—	—	+
Th	Cos	*Sonchus asper*	—	—	—	—	+
Th	Eua	*Malva neglecta*	—	—	—	—	+
Th	Eu	*Adonis aestivalis*	+	+	+	+	+
H	Eua	*Cichorium intybus*	—	—	—	—	—
Th	Eua	*Galium aparine*	+	+	+	+	+
H	Eua	*Stellaria graminea*	+—1	+	+	+	+
Th	Cpl	*Polygonum convolvulus*	—	—	—	—	—
H	Eu	*Rumex obtusifolius*	—	—	—	—	—
Th	Cos	*Amaranthus retroflexus*	+	—	+	—	—
H	Eua	*Plantago major*	—	—	—	—	—
Th	Cos	*Centaurea cyanus*	+	+	+	—	—
Th	Eua	*Descurainia sophia*	+	+	+	—	—
H	Cos	*Sonchus arvensis*	+	+	—	—	—
Th	Eua	*Lepidium draba*	+—1	+	+	+	+
Th	Eua	*Matricaria chamomilla*	+	+	—	+	+
Th-TH	Eua	*Matricaria inodora*	+	+	+	+	+
H	Eu	*Ranunculus sardous*	+	+	—	—	+
Th	Cos	*Chenopodium album*	+	—	—	—	+
Th	Eua	*Lithospermum arvense*	+	+	—	—	—

of Lotus corniculatus 1973—1976

1974				1975				1976			
3. V.	20. VI.	8. VII.	28. IX.	21. V.	20. VI.	15. VII.	25. IX.	11. V.	15. VI.	24. VIII.	23. IX.
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
90	80	90	85	80	80	80	80	85	85	90	90
2	2	2	2	1–3	1–3	1–3	1–3	3	3	3	3
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
–	–	+	+	1	1	1–3	1–3	1–2	1–2	1–3	1–3
+	+	+	–	–	+	+	–	–	–	–	–
+	–	–	–	–	–	–	–	–	–	–	–
+	–	–	–	–	–	–	–	–	–	–	–
+	+	+	+	+	+	+–1	1–2	+–2	+–2	1–2	1–2
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	1–3	+–3	+–3	+–2	+–2			+–2
+	1	+	+	+	+	+	1	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	1	+	+	+	1	+–2	+–2	+–1	+–1	+–1	+–1
–	–	+	+	+	+	+	+	+	+	+	+
–	–	+	+	+	+	1	1	+–1	+–1	+–1	+–1
+	+	+	+	+	+	+	+	–	–	–	–
–	–	–	–	+	+	+	+	–	–	–	–
–	–	–	–	–	–	–	–	+	+	+	+
–	–	–	–	–	–	–	–	+	+	+	+
–	–	–	–	–	–	–	–	+	+	+	+
1–3	1–3	1–3	1–2	+–1	+–1	+–1	+–1	+–1	+–1	+–1	+–1
+–3	1–2	1–2	+–2	1	1	1	1	+	+	+	+
+	+	+	+	+	+	+–2	+–1	+–1	+–2	+–2	+–3
+	+	+	+	+	+	+	+	+	+	1–2	1–2
+	+	+	1	1	+	+	+	+	+	+	+
+	+	1	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+–1	+–1	+	+
+	+	–	–	+	+	+	+	+–1	1–2	1–2	1–2
+–1	+	+	+	+	+	+	+	–	–	–	–
+	+	+	+	+	+	+	+	+–1	+–1	1–2	1–2
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	–	–	–	–	–	+	+	+	+
–	+	+	+	+	+	+	+	+	+	+	+
+	+	+	–	–	+	+	–	–	–	–	–
+	–	–	+	–	+	+	+	–	–	–	–
+	+	–	–	–	+	+	+	+	+	+	+
–	–	–	+	+	+	+	+	+	+	+	+
+	+	–	–	–	–	–	–	+	+	+	+
–	–	–	–	+	+	+	+	+	+	+	+
+	+	+	–	–	+	–	–	–	–	–	–
+	+	+	–	–	+	–	–	–	–	–	–
+	–	–	–	–	–	–	–	+	+	+	+
+	–	–	–	–	–	–	–	–	–	–	–
+	+	+	+	+	–	–	–	–	–	–	–
+	–	–	–	–	–	–	–	–	–	–	–
+	+	+	–	–	–	–	–	–	–	–	–
+	+	–	–	–	–	–	–	–	–	–	–
+	+	–	–	–	–	–	–	–	–	–	–

Date of survey			1973				
			3. V.	5. VI.	5. VII.	28. VIII.	3. X.
Growth			1st	2nd	3rd	4th	5th
General cover, %			100	100	90	90	90
Th	Cos	*Portulaca oleracea*	—	—	—	—	—
Th	Cos	*Hibiscus trionum*	—	—	—	—	—
H	M	*Ballota nigra*	—	—	—	—	—
Th	Adv	*Erigeron canadensis*	—	—	—	—	—
H	Cos	*Prunella vulgaris*	—	—	—	—	—
Th	Eua	*Camelina microcarpa*	+	—	—	—	—
TH	Eua	*Conium maculatum*	+	+	—	—	—
Th	Cos	*Chenopodium hybridum*	—	—	—	—	+
Th	M	*Consolida orientalis*	—	—	—	—	+
Th	Cos	*Sinapis arvensis*	+	+	—	—	—
Th	M	*Stachys annua*	—	—	—	—	+
Th	Cos	*Papaver dubium*	+	+	—	—	—
Th	Eua	*Papaver rhoeas*	+	—	—	—	—

species doubled (40), though their weight ratio in the total yield fell to 4.6%. In 1975 the stand of the species sown became thinner, so the invader species, with the same number of species as in the previous year, increased their populations, and their weight ratio in the total crop rose to 9.8%. By the end of 1976 the *Festuca pratensis* almost completely disappeared, to be replaced by other species, of which *Dactylis glomerata* and *Trifolium repens giganteum* had a value of 1—2 and *Taraxacum officinale*, *Convolvulus arvensis*, *Geranium pusillum*, *Stellaria media* and *Crepis setosa* a value of 1—3 on the Braun–Blanquet scale. The weight percentage of non-grass species was as high as 36.5% (Table 4).

In four years 55 species were observed in the trial plots. The number of invader species was the highest in spring and decreased by the autumn. The same applies to their proportion in the crop. The serious degradation of the grass, the extent to which it became overgrown by weeds, is shown by the ecological and area spectra of the first and fourth years.

Ecological spectrum:

1973: Th-64%, H-24%, H-G-4%, G-4%, Th-TH-4%
1976: Th-43.9%, H-39.02%, Th-TH-9.76%, G-4.88%, H-G-2.44%

Area spectrum:

1973: Cos-44%, Eua-32%, Eu-8%, Adv-8%, Cpl-4%, M-4%
1976: Eua-43.9%, Cos-31.7%, Eu-7.32%, Adv-7.32%, Cpl-4.88%, K-2.44%, M-2.44%.

The rapid thinning of the grass stand sown with *Festuca pratensis* can be explained by the compactness of the soil caused by excessive irrigation, together with the stabilization of more aggressive species with higher viability. The green crop of the stand on the average of four years was 580.95 q/ha (143.9 q/ha dry matter), but its feeding value, especially in the last two years, was lower than that of similar, well-established grasses.

3. *Bromus inermis* stand. In order to develop a grass stand of 33,000 plants per 100 m^2, sowing had to be carried out twice in 1973. It was only the vigorous growth and strong habit of this species that enabled a 50—60% cover to be attained by the end of the first vegetation period. In the year of plantation an average of 19—28 strange species occurred in the grass with a cover of 15—20%. They made up 15.33% by weight of the total crop. In 1974 the species sown achieved a 70% cover; the number of invader species doubled (40—44), but their weight

1974				1975				1976			
3. V.	20. VI.	8. VII.	28. IX.	21. V.	20. VI.	15. VII.	25. IX.	11. V.	15. VI.	24. VIII.	23. IX.
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
90	80	90	85	80	80	80	80	85	85	90	90
−	−	−	−	+	+	+	+	−	−	−	−
−	−	−	−	−	−	−	−	+	+	+	+
−	−	−	−	−	−	−	−	+	+	+	+
−	−	−	−	−	−	−	−	+	+	+	+
−	−	−	−	−	−	−	−	+	+	+	+
+	−	−	−	+	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−
+	+	−	−	−	−	−	−	−	−	−	−
+	+	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−
+	+	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−
+	+	−	−	−	−	−	−	−	−	−	−

ratio in the total yield fell to 5%. In 1975, after the first growth had been cut, the population of the species sown began to thin out. The number of invader species was 43—46, of which *Dactylis glomerata*, *Festuca pratensis*, *Lolium perrenne*, *Trifolium repens giganteum*, *Stellaria media* and *Lamium amplexicaule* occurred to the greatest extent. The proportion of invader species in the total crop, expressed in dry matter, amounted to 9.62% by weight. In 1976 the stand of the species sown was further degraded, and the number and populations of the mainly perennial invader species became constant. The proportion of invader species in the total yield reached 38.7% by weight expressed in dry matter (Table 5).

When considering the number of species in the successive growths it was found to increase steadily until the end of the third year and to become more or less stable in the fourth year. As to the weight percentage of invader species in the total crop, it was always the highest in the first growth and decreased by the autumn.

In four years 58 species were found in the trial plots. The ecological and area spectra of the first and fourth years also reflect the process of degradation.

Ecological spectrum:

1973: Th-57.15%, H-28.57%, Th-TH-10.71%, G-3.56%
1976: Th-48.72%, H-38.46%, Th-TH-7.69%, G-5.13%.

Area spectrum:

1973: Eua-60.72%, Cos-32.14%, Eu-3.57%, K-3.57%.
1976: Eua-41.03%, Cos-33.34%, M-10.26%, Eu-5.12%, Adv-5.12%, Cpl-2.56%, K-2.56%.

In four years the species sown gradually became thinner and was replaced by grasses, papilionaceous plants and mainly perennial species from the neighbouring grassfields. The structural degradation of the stand was caused by the compactness of the soil and the abundant water supply. On the average of four years the green crop of the grass sown amounted to 628.83 q/ha (154.28 q/ha dry matter), but owing to the species composition the feeding value was lower than that of similar single-species grasses.

4. Grass stand of *Dactylic glomerata* L. From 310 g seed sown over an area of 100 m^2 an average of 127,000 seedlings developed. In the very first year a vigorous, sufficiently closed

stand evolved, in which the species sown covered 80—85% of the area. The number of mainly annual invader species was negligible (15—18), with a cover of 3—5% and a proportion of 4.51% by weight in the total crop. In 1974, in spite of a slight increase in the number of species (19) the weight ratio of the invader species remained almost unchanged (3.45%). In 1975 the stand of the species sown began to grow thinner and the annual species were replaced by perennial ones, though the number of species remained the same (18—20). The weight ratio of the invader species doubled (6.85%). In 1976 the stand of the species sown continued to thin out, giving a 40—50% cover. The weight percentage of the invader species, including valuable grasses and papilionaceous plants, reached 38.3% in dry matter, as the species number was also twice as high as in the previous year (27—29). Of the successive growths the first was always most severely affected by weeds; by the autumn the situation improved (Table 6).

In four years 37 species were found in the trial plots, the lowest number for all the grasses included in the trial. The ecological and area spectra of the first and fourth years also reflect the gradual degradation of the grass and the process of species replacement .

Ecological spectrum:

1973: Th-55%, H-25%, H-G-5%, TH-5%, Th-TH-5%
1976: Th-55.17%, H-34%, H-G-3.45%, G-3.45%, Th-TH-3.45%

Area spectrum:

1973: Eua-60%, Cos-40%
1976: Cos-44.83%, Eua-34.48%, Cpl-6.89%, Eu-3.45% K-3.45% M-3.45%, Adv-3.45%.

The rapid degradation of the grass stand sown with *Dactylis glomerata* can be explained by the invasion of more vigorous perennial species and the compactness of the soil caused by excessive irrigation. The green crop of the grass sown was 640 q/ha (145 q/ha dry matter) on the average of four years.

5. Grass stand of *Trifolium repens* L. f. *giganteum* Lagr. From 170 g seed sown in the 100 m^2 trial plots an average of 112,000 seedlings came up. By the end of the first year a vigourously developing and sufficiently closed stand was produced in which the species sown attained a 60—70% cover. The number of other, mainly annual species was 15—24; they covered 10—15% of the surface and their weight ratio in the total crop was 20%. In 1974 the number of species in the successive growths was as high as 27—35, but their weight ratio was so insignificant that weighing was discontinued. *Stellaria media* and *Lamium amplexicaule* occurred in the largest individual numbers in the stand; with their short stems they developed well under the species sown. In 1975 the annual species were replaced here too by perennial ones. The number of species was smaller (25—32) than in the previous year, as was the individual number. In 1976 the species sown developed a pure stand, in which quite a lot of other species (19—21) were to be found, but with a low number of individuals, so they covered hardly 2% of the surface (Table 7).

In four years 51 species were found in the trial plots, though in relatively low individual numbers. The gradual replacement of species in the stand is shown by the ecological and area spectra of the first and fourth years.

Ecological spectrum:

1973: Th-62.51%, H-25%, Th-TH-6.25%, H-G-3.12%, G-3.12%
1976: Th-41.67%, H-41.67%, Th-TH-8.34%, H-G-4.16%, G-4.16%.

Area spectrum:

1973: Eua-53.14%, Cos-34.38%, Cpl-3.12%, Eu-3.12%, M-3.12%, Adv-3.12%
1976: Eua-50%, Cos-29.18%, Adv-8.34%, Eu-4.16%, Cpl-4.16%, M-4.16%.

By the end of the fourth year the stand formed a completely closed, thick grassfield, since this variety quickly adapts itself to both an abundant water supply and an alkaline soil. The green crop of the stand was 694.6 q/ha (140.6 q/ha dry matter) on the average of four years.

6. *Lotus corniculatus* L. stand. From the seeds sown on 13th March 1973 a very thin population developed, and planting was therefore repeated on 31st August 1973. Even this resulted in only 5000 seedlings on 100 m². Since the proportion of *Lotus corniculatus* in the stand was insignificant, the production test was omitted and only cenological observations were made.

In four years the initial annual species, of which *Lamium amplexicaule*, *Stellaria media*, *Cerastium caespitosum*, *Stellaria graminea* and *Lepidium draba* displayed the largest individual numbers and cover, were mostly replaced by perennial grasses, papilionaceous plants and other perennial species. During this period the population of *Lotus corniculatus* L., a species which rapidly adapts itself to alkaline soil and abundant water supply, also increased, mainly by seeding, and by the end of the fourth year it covered 40—50% of the surface, compared to 10—20% in the previous year (Table 8).

Of the stands examined the largest number of species (64) was found in this one. From the second year on, in spite of a continuous replacement of species, an average of 40 species occurred in the 100 m² plots in a highly varying population composition. The rapid change of the individual populations is also shown by the ecological and area spectra of the first and fourth years.

Ecological spectrum:

1973: Th-62.5%, H-25%, Th-TH-5%, H-G-2.5%, G-2.5%, TH-2.5%
1976: H-48.73%, Th-38.46%, G-5.13%, H-G-2.56%, Th-TH-2.56%, HH-2.56%.

Area spectrum:

1973: Eua-45%, Cos-45%, Eu-5%, M-2.5%, Adv-2.5%
1976: Eua-35.9%, Cos-35.9%, Cpl-7.69%, Eu-7.69%, M-5.13%, Adv-5.13%, K-2.56%.

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Prepared at the Department of Phytotaxonomy and Ecology of the Eötvös Loránd University, Budapest.

A. Kovács

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UREA ALDEHYDE CONDENSATION PRODUCTS AS FERTILIZERS AND THEIR EFFECT ON CALCAREOUS SANDY SOILS

The introduction of heavy dressing has resulted in considerable yield increases all over the world. This fact has called for the use of some new fertilizer types which would not harmfully affect the development of plants even if applied in high doses; morever, due to their "retarded" nutrient release, they would promote steady plant growth and the achievement of reliable high yields.

As early as in 1923 Blanck and Giesecke (BLANCK—GIESECKE 1923) called attention to the application of urea-aldehyde condensates as nitrogen fertilizers. Compounds of this type were called "Ureaforms" by CLARK *et al.* (1948). According to their different aldehyde components the products were put on the market under different names: Nitroform, Floranid, IBDU, Urea-Z, etc. (KRALOVEC—MORGAN 1954, SCHEFFER *et al.* 1957, JUNG 1961, MIKES—GÁTI 1966, HAMAMOTO 1966).

The production of the simplest ureaform fertilizers is based on the reaction of urea with formaldehyde and, depending on the different conditions of the reaction (pH, urea-formaldehyde ratio, condensation degree, temperature, etc.), several compounds are produced (DE JONG—DE JONGE 1952, TATSUO HAYASE 1959, 1960, ALMÁSSY 1973). HAYS (1963) qualified the fertilizers on the basis of their N-supplying capacity, characterized by the so-called "activity index".

It is only in some cases that the experimental results have justified the yield-increasing effect of ureaform products and, compared with water soluble N fertilizers, the accumulation of nitrogen incorporated into plants was generally also inferior. ANSORGE (1955, 1962) conducted pot and field experiments with ureaform products of different solubilities, using rye-grass, oat, rye, maize and sunflower. The condensates had the same effect as calcium ammonium nitrate. In their experiments SCHMALFUSS—MICHAEL (1956) established the favourable response of mustard to ureaforms. According to ATANASIU (1959) the nitrogen supply of potato, oat and wheat could be ensured for the second year, too. In the course of his field experiments with oat, potato and celery KNOP (1965) observed that the effect of ureaforms increased with the rise of the urea-formaldehyde mole ratio. GÁTI (1967) carried out field experiments with Sudan grass and found a correlation between the crop yield and the solubility of the condensates; mixtures of condensates and ammonium nitrate gave the best results. MÁRTA (1976) reported favourable responses of ornamental plants (*Gladiolus cultorum* Domk. and *Scindapsus aureus* Lind et André Engl.) to ureaform fertilizers.

The application of urea-formaldehyde condensation compounds as fertilizers is promising mainly on light textured sandy soils, in which the nutrients, especially N, are rapidly leached. For this reason the favourable effect of slow acting fertilizers can be expected on these soils.

The present paper is intended to give a short account of experiments conducted for more than 10 years on a slightly humous calcereous sandy soil in Hungary. The effects of several ureaform products of different activities were compared in pot experiments with rye-grass *(Lolium perenne)*. The results of a 5-year experiment with Sudan grass, wheat and rye are evaluated as well.

a) Pot experiments were carried out at three N levels using ureaform products of nearly equal total nitrogen contents (40.5—40.8%) N and different activities (A_i = 0, 17, 29, 38 and 51). The total N-contents and activities of the condensates compared are presented in Table 1. The ureaforms applied were the products of the Budapest Chemical Works (Budapesti Vegyiművek).

The activity index (A_i) was calculated according to the formula given in the Official Methods of Analysis of the A.O.A.C. (ANONYMOUS 1960)

Table 1

Characteristics of the condensates

Sign of the condensate	Activity, A_i	Total N, %	CWIN	HWIN
			%	
UF_1	∅	40.7	31	30.9
UF_2	17	40.4	34.7	28.7
UF_3	29	40.7	32.2	22.8
UF_4	38	40.5	30.5	18.9
UF_5	51	40.8	29.6	14.3

$$A_i = \frac{\%\,CWIN - \%\,HWIN}{\%\,CWIN}\,100$$

where

CWIN = cold water insoluble N
HWIN = hot water insoluble N

Besides basic PK fertilization (300 mg P_2O_5 and 400 mg K_2O/2 kg soil in each pot) N was added at 3 levels ($N_1 = 100$ mg N, $N_2 = 200$ mg N and $N_3 = 400$ mg N/pot); the indicator plant was rye-grass *(Lolium perenne)* in slightly humous, calcareous sandy soil.

Four times in the course of the vegetation period, at intervals of 4—6 weeks the plants and shoots were cut to determine the dry yields and the NPK contents of the plants after crushing with $H_2SO_4 + H_2O_2$.

Table 2

Treatments and characteristics of the applied fertilizers

Treatments	Characteristics of the fertilizers
1. Superphosphate (18% P_2O_5) and potassium salt (40% K_2O)	
2. Péti-salt (Nitro-Chalk)	Water soluble N = 25%
3. Urea	Water soluble N = 46%
4. Nitroform (USA)	Condensation product urea-formaldehyde Total N = 40% $A_i = 50$
5. Floranid (GFR)	Condensation product urea-crotonylidene aldehyde Total N = 27.1% $A_i = 100$
6. IBDU (Japan)	Condensation product urea-isobutyridene aldehyde Total N = 30.5% $A_i = 97$
7. Varioform I (Hungary)	NH_4NO_3, condensed with 10% aminoplast synthetic resin solution Total N = 33.2%
8. Varioform II (Hungary)	Urea, condensed with 10% aminoplast synthetic resin solution Total N = 43.1%
9. Monipol (Hungary)	60% NH_4NO_3, 31.5% bentonite, 1.7% $MgSO_4$, condensed with 6.8% aminoplast synthetic resin solution Total N = 26.2%
10. Monit (Hungary)	65% NH_4NO_3, 33.0% bentonite and 2% $MgSO_4$ mixture Total N = 23.4%

b) In the 5-year field experiment the fertilizing effects of different Hungarian (Varioform I and II, Monipol) and foreign (Nitroform, Floranid, IBDU) urea condensation products were compared with that of Péti-salt (Nitro-Chalk) and urea (Table 2).

The experiments were conducted in the years 1967—1972 at Őrbottyán (Experimental Farm of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences) on a slightly humous calcareous sandy soil: $pH_{(H_2O)} = 7.6$; $pH_{(KCl)} = 6.9$; $CaCO_3 = 5.4\%$; Humus = 0.74%; $hy_1 = 0.89$. Total-N, AL-soluble P_2O_5, AL-soluble K_2O were 39.2 mg, 3.3 mg, 6.6 mg/100 g soil respectively.

In the course of four years (1967—1971) the fertilizers were applied in two doses:

$N_1P_1K_1$ = 100 kg, N, 35 kg P_2O_5 and 40 kg K_2O/ha
$N_2P_2K_2$ = 200 kg N, 70 kg P_2O_5 and 80 kg K_2O/ha

In 1972 the treatment was uniformly 35 kg P_2O_5 and 40 kg K_2O/ha without nitrogen. As P-source superphosphate (18% P_2O_5) and as K-source potassium-salt (40% K_2O) were used in all the experiments.

Crop sequences were: Sudan grass (1968), rye (1969), winter wheat (1970 and 1971) and Sudan grass (1972).

Further data: plot size = 26 m^2, 5 replications, split-plot and Latin square design.

For the evaluation of the experimental results the dry yield (86% dry matter) of Sudan grass and the grain + straw yield of cereals were considered.

I. Pot experiments with ureaform products of different activities

Dry matter yields and data of nutrient uptakes for the different shoots, as a function of N levels and activities, are presented in Figure 1. Studying this figure (a, b, c and d) we are able to follow the action process of ureaform fertilizers and the effect of activity in its dynamics.

It can be observed that within the same activity but also independently of the activity, the dry matter yields and nutrient uptakes were in general positively affected by increasing the N rates in each fertilizer form. In the case of urea there was a depression at the N_3 level as compared with the N_1 and N_2 levels. This fact indicates the toxic effect of high rate urea at the early stage of plant development; this depressing effect was not present with ureaforms.

At the same time, dry yields and nutrient uptake increased proportionally with the increased activity. In the treatment with urea the dry yield became minimum in the 4th shoot (Fig. 1a); in the case of ureaforms of higher activities (A_i = 38 and 51) the dry yields were quite high.

This tendency also prevailed in the N uptakes which were favourably affected by the increased activity (Fig. 1b). The process of P uptake proved the superiority of ureaforms as compared with urea. Using ureaform preparates with activities of A_i = 38 and 51, P uptake was outstandingly high, especially at the N_3 level, as seen clearly in Fig. 1c. K uptake shows a similar increasing tendency with ureaforms of higher activities; with urea, however, a certain depression could be observed in the 1st and 4th shoots at the N_3 level (Fig. 1d).

A study of the action process indicates that the N-supplying capacity of ureaforms increases proportionally with the increase in their activities; this fact is reflected positively by the increase of dry matter. Ureaforms advantageously affected P and K uptakes, too.

The summarized yield data of the above-mentioned greenhouse experiments and the NPK amounts taken up are presented in Table 3.

Positive relationships were found between the increase in the dry yield, the activity index and the N, P and K amounts taken up by the plants. At the N_1 level the dry yield in-

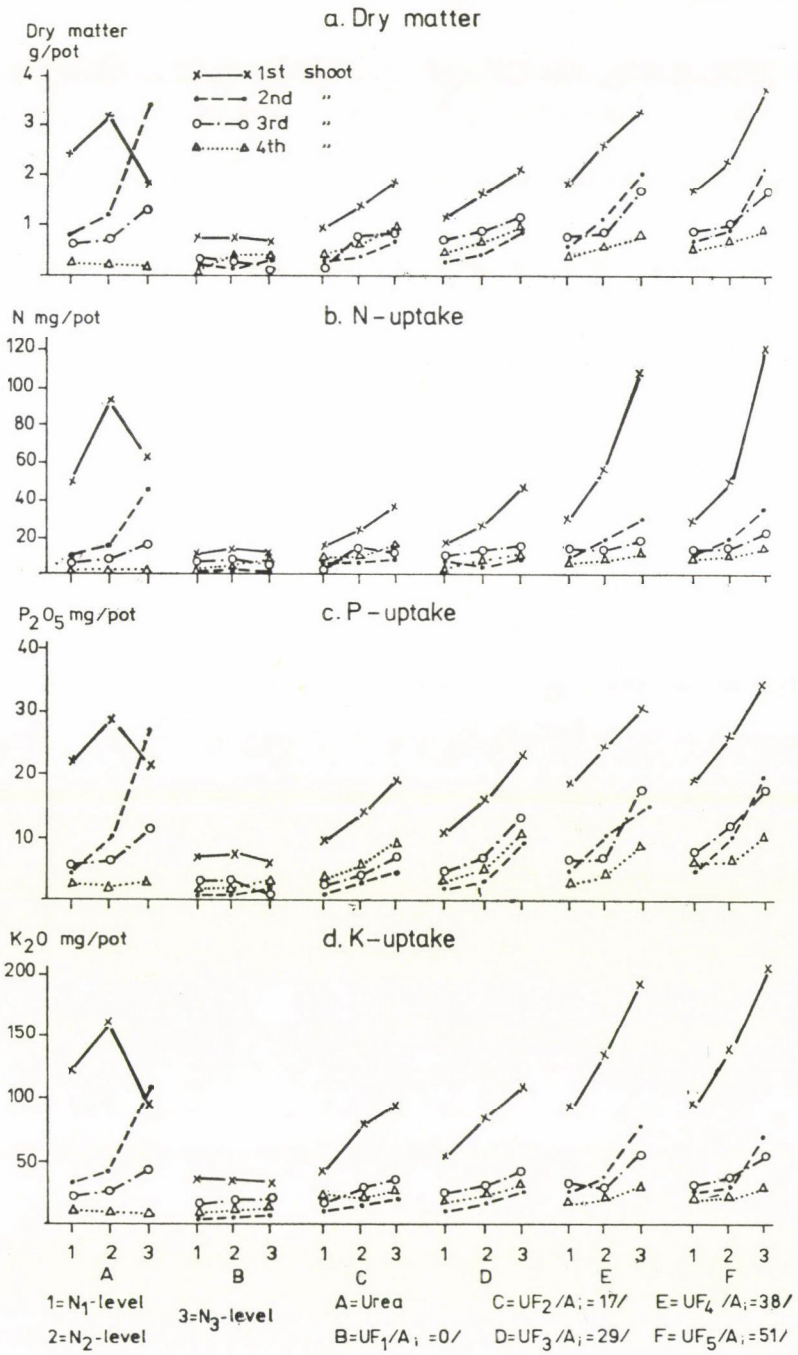

Fig. 1. Dry matter yields and nutrient uptake as a function of N levels and activities

Table 3

Dry matter yield and N, P, K uptake of rye-grass (Lolium perenne) (Averages of 4 shoots)

Treatments		Dry matter, g/pot					Assimilated N, mg/pot				
No.	Fertilizer form	—	N_1	N_2	N_3	$LSD_{5\%}$	—	N_1	N_2	N_3	$LSD_{5\%}$
1	PK	1.39					21.3				
2	Urea		3.97	5.10	6.80			71.2	121.6	130.7	
3	UF_1 (A_i = ∅		1.32	1.47	1.32			17.9	20.1	18.8	
4	UF_2 (A_i = 17)		2.12	3.12	4.31	0.19		33.0	51.6	78.5	4.4
5	UF_3 (A_i = 29)		2.41	3.45	4.93			36.7	53.9	89.8	
6	UF_4 (A_i = 38]		3,51	5,04	8,00			58,4	95,4	183,8	
7	UF_5 (A_i = 51)		3.31	4.76	8.08			55.7	8.8	194.7	
	$LSD_{5\%}$			0.47					10.7		

Treatments		Assimilated P_2O_5, mg/pot					Assimilated K_2O, mg/pot				
No.	Fertilizer form	—	N_1	N_2	N_3	$LSD_{5\%}$	—	N_1	N_2	N_3	$LSD_{5\%}$
1	PK	13.9					52.2				
2	Urea		36.1	45.9	63.3			165.7	225.2	268.1	
3	UF_1 (A_i = 0)		12.8	14.7	12.8			49.5	53.6	48.8	
4	UF_2 (A_i = 17)		25.5	32.0	46.0	1.79		80.5	128.2	187.7	5.8
5	UF_3 (A_i = 29)		25.1	35.7	55.9			95.4	139.6	212.1	
6	UF_4 (A_i = 38)		36.7	50.0	78.7			151.3	228.4	360.9	
7	UF_5 (A_i = 51)		38.0	55.9	83.1			139.6	227.4	368.1	
	$LSD_{5\%}$			4.39					14.3		

creasing effect of urea surpasses that of the condensates. At the N_2 level the differences in the dry yields, compared with urea, in the cases of A_i = 38 and 51 remain within the limits of significance. At the N_3 level significantly higher yields were obtained with these two condensates than with urea.

The same tendencies were observed for N, P and K uptakes, too. The availability of condensates with a higher activity index significantly surpassed that of urea. The condensates also exerted a favourable influence on P and K mobilisation. At all three N levels the amount of P deriving from the fertilizer increased in a direct ratio to the increased activity. At the N_2 level the condensates and urea had the same effect on the P and K uptakes, whereas at the N_3 level the condensates proved to be significantly superior.

Our earlier opinion on the favourable effect of the condensates was supported by the data obtained concerning the recovery of nutrients. The recovery of nutrients indicates quantitatively the rate of uptake of N, P and K deriving from the fertilizers and is expressed as a percentage (Table 4).

Table 4

Recovery of nitrogen, phosphorus and potassium

Treatments	N-recovery, % N-level			P_2O_5-recovery, % N-level			K_2O-recovery, % N-level		
	N_1	N_2	N_3	N_1	N_2	N_3	N_1	N_2	N_3
Urea	49.9	50.1	27.3	7.4	10.7	16.4	28.4	43.2	54.0
UF_1	—	—	0.1	—	0.3	—	—	0.3	24.1
UF_2	11.6	15.1	14.3	3.9	6.3	10.7	7.1	18.9	33.6
UF_3	16.5	16.3	17.1	3.7	7.2	13.9	10.8	21.8	39.9
UF_4	37.1	37.0	40.6	3.6	12.0	21.6	24.8	44.0	77.2
UF_5	34.3	33.7	43.3	8.0	14.0	23.0	21.8	43.8	79.0

As shown by the data, the recovery of nutrients increased with the activity. It is only at the N_3 level that the recovery from the two condensates with the highest activities surpasses that from urea. As regards the uptake of P and K, positive effects were already observed at the N_2 level. At the N_3 level the recovery of P and K was more favourably influenced by these condensates than by urea.

It is known that from among the fertilizer nutrients P displays the least availability, partly due to its fixation in the soil. Since the recovery of P was favourably affected by condensates with high activity, we supposed that a regular N supply promoted the uptake of P from the soil. K recovery was the most favourable, reaching as much as 79% with high activity condensates at the N_3 level. N recoveries were: 43.3% from the product at $A_i = 51$ and 27.3% from urea.

The data indicate that dry matter yield and nutrient uptake were favourably influenced by ureaform fertilizers in proportion to their activity. The release of the N content of the condensate and its incorporation into the vegetal organism also depends on the activity. It was further established that, due to the regular N supply ensured by the condensates, the K and P nutrition of the plants was promoted.

II. Field experiments

In Table 5 the crop yields and surplus yields obtained (calculated in grain units = GU) are given.

Special attention should be paid to the results obtained with treatments 4 to 8 and 10, i.e. with condensates with a urea-formaldehyde base. In the first year (1968), with the exception of Varioform II (treatment 8), the condensates gave lower yields than Péti-salt and urea. In the second year (1969) Floranid, IBDU and Varioform II seemed to be the most effective at the N_{200} level. Using lower rates (N_{100}) higher yields were observed, due to residual effects, with Varioform I in the third year (1970), as well as with several condensates (Nitroform, Floranid and IBDU) in the fifth year. At a higher N level (N_{200}) Varioform II seemed to be more effective in the fourth year (1971), and Nitroform, Floranid, IBDU, Varioform I and Monipol in the fifth year (1972) as compared with Péti-salt and urea. When considering the total yields obtained during the whole vegetation period it can be established that, besides Péti-salt, IBDU was effective at the N_{100} level; at the N_{200} level IBDU and Varioform II were superior to Péti-salt and urea. The same tendencies were observed concerning yield sur-

Table 5

Yields and the same expressed in grain units (GU) 1968—1972

Treatments		Yields, q/ha					Grain unit (GU), q/ha	
No.	N-level	Sudan grass dry matter 1968	Rye grain[1] 1969	Winter wheat grain 1970	Winter wheat grain 1971	Sudan grass dry matter 1972	Total yield[2] 1968—72	Surplus as compared with control
1		12.07	5.71	0.83	4.51	40.16	34.46	—
2		22.62	11.74	2.25	16.99	51.46	66.83	32.37
3		25.26	11.64	1.79	18.64	40.08	64.19	29.73
4		14.01	10.16	1.80	13.17	54.56	56.88	22.42
5	N_{100}	14.78	10.90	1.62	12.88	56.98	58.57	24.11
6		16.43	12.38	1.37	17.00	58.28	66.58	32.12
7		21.12	11.21	2.57	16.79	52.46	65.86	31.40
8		28.18	10.47	1.93	14.99	48.74	63.41	28.95
9		24.87	13.43	2.11	17.42	43.92	66.96	32.50
10		20.83	10.69	1.28	13.52	43.40	55.97	21.51
1		11.06	5.29	0.97	6.12	40.70	35.81	—
2		23.55	11.42	2.70	22.65	40.68	70.52	34.71
3		36.35	13.96	2.77	22.02	34.50	74.78	38.98
4		20.08	12.27	2.38	19.63	58.44	71.78	35.97
5	N_{200}	16.51	16.40	2.12	20.18	55.56	74.57	38.76
6		22.14	15.87	2.26	23.31	68.74	85.44	49.63
7		30.06	12.69	2.39	19.86	45.78	72.51	36.70
8		35.64	15.76	2.63	24.55	43.00	83.09	47.28
9		30.17	14.39	2.71	19.27	46.22	74.01	38.20
10		29.14	13.65	3.07	17.83	49.72	73.03	37.22
	$LSD_{5\%}$	6.51	2.35	1.13	4.08	15.38	17.97	

1 = The yield was extremely low as a consequence of dry weather without precipitation.
2 = During the 1968—72 period the whole yield was expressed in grain units (GU), which in cereals include the grain and straw yield. In Sudan grass the conversion factor for grain units = 0.4; in the straw yield of cereals it is 0.16.

pluses, too. In general, it was established that slow acting condensates have cumulative nitrogen effects and especially the responses to IBDU were very favourable at both nutrient levels. At higher nutrient levels good results were obtained with Varioform II as well.

Pot and field experiments were carried out to study the effect of urea aldehyde condensates as N fertilizers on a slightly humous calcareous sandy soil.

In pot experiments the response of rye-grass (Lolium perenne) to ureaform compounds of different activities (A_i = 0, 17, 29, 38 and 51) were compared at 3 N levels (N_1 = 100 mg N, N_2 = 200 mg N and N_3 = 400 mg N/2 kg soil) and on 4 shoots. At the N_3 level urea had a depressing effect on the yield of the first shoot; this unfavourable influence was not observed

with ureaforms. The response to urea decreased after each cut, that to ureaforms proved to be favourable depending on their activities. The total yield data of four shoots indicated that at the highest level (N_3) condensates with activities of 38 and 51 gave significantly more favourable results than urea. This tendency was also observed with reference to the amount of N taken up by the plant. P and K uptake was also favourably affected by ureaforms of higher activities.

The field experiments were conducted with Sudan grass, rye and wheat and the effects of Nitroform (USA), Floranid (GFR) and IBDU (Japan) urea aldehyde condensates, as well as those of Varioform I and II and Monipol (Hungary) condensates on an aminoplast base were compared with those of Péti-salt and urea. Over the years the cumulative N effect of the condensation products was established; IBDU and Varioform II seemed to be superior.

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Prepared at the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest.

F. GÁTI

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CHANGES IN THE ZINC AND TRYPTOPHANE CONTENTS OF MAIZE GRAINS AS A RESPONSE TO INCREASING RATES OF PHOSPHORUS FERTILIZATION

In a field trial carried out on brown forest soil, phosphorus applied at increasing rates was found to decrease the zinc and tryptophane contents in various parts of the maize grain. The decrease in the tryptophane content reduced the biological value of the maize. The increasing use of high rate phosphorus fertilization on this type of soil makes it necessary to experiment with the zinc nutrition of maize.

The maize grain was separated into two parts: germ and endosperm, and the zinc and tryptophane contents were determined in each. The zinc was determined by polarography and the tryptophane by photocolorimetry with *p*-dimethyl-amino-benzaldehyde as the reagent in a highly acidic medium without hydrolysis.

The field trial was set up on brown forest soil (at Keszthely) with carbonate loess loam as parent material. The trial was a long-term experiment; the examinations were performed 12 years after the experiment was set up. The number of replications was 6. The laboratory analyses were made on samples taken from 4 series, with 12 ears per plot.

The plant used in the trial was MvSC 530 hybrid maize.

The pH of the soil in water was 7.4; its humus content was 2.0%.

The mobile potassium content determined by the AL method was 10 mg/100 g soil.

The soil samples were taken in July.

The treatments used in the trial were as follows:

Treatment	N	K_2O	P_2O_5	AL-P_2O_5, mg/100 g soil	Yield, q/ha
	kg/ha				
AP/1	—	—	—	2	48.0
AP/2	175	140	—	2	57.6
AP/3	175	140	70	3	67.1
AP/4	175	140	140	6	72.1
AP/5	175	140	210	17	68.9
AP/6	175	140	280	18	69.5

The yield data are the averages of six replications.

Table 1

Data characteristic of the weight conditions of components in the maize grain in a phosphorus fertilization trial

Treatment	Grain weight, mg	Germ weight, mg	Germ, %	g/es* relation
I/1	238.4	24.8	10.40	0.1156
II/1	233.4	23.9	10.24	0.1145
III/1	216.5	20.8	9.61	0.1100
IV/1	245.3	26.1	10.64	0.1183
Average:	233.4	23.9	10.22	0.1146
I/2	261.2	25.7	9.84	0.1158
II/2	256.3	26.1	10.18	0.1155
III/2	276.2	27.3	9.88	0.1134
IV/2	271.2	27.7	10.21	0.1165
Average:	266.2	26.7	10.03	0.1153
I/3	295.2	32.4	11.00	0.1227
II/3	288.6	30.0	10.40	0.1224
III/3	279.6	27.2	9.74	0.1114
IV/3	261.4	27.5	10.53	0.1175
Average:	281.2	29.3	10.40	0.1185
I/4	287.6	32.0	11.13	0.1248
II/4	275.4	30.4	11.04	0.1225
III/4	272.6	30.0	11.00	0.1194
IV/4	265.9	29.3	11.02	0.1234
Average:	275.4	30.4	11.15	0.1225
I/5	286.9	30.3	10.56	0.1180
II/5	291.4	30.5	10.47	0.1257
III/5	285.9	32.3	11.30	0.1269
IV/5	298.3	33.3	11.16	0.1242
Average:	290.6	32.1	10.87	0.1237
I/6	305.7	34.1	11.15	0.1257
II/6	275.5	29.4	10.67	0.1237
III/6	281.7	31.3	11.11	0.1287
IV/6	287.6	31.6	11.00	0.1260
Average:	287.6	31.6	10.98	0.1260
$SD_{5\%}$	16.5	2.6	0.53	0.0052

* Germ/endosperm.

Table 2

Zinc and tryptophane concentrations in the components of maize grain in a phosphorus fertilization trial

Treatment	Zinc, ppm		Tryptophane, g/100 g air-dry matter	
	germ	endosperm	germ	endosperm
I/1	156.8	6.0	0.196	0.0402
II/1	140.0	5.0	0.181	0.0400
III/1	148.0+	7.2+	0.189+	0.0348+
IV/1	148.8	5.5	0.189	0.0404
Average:	148.5	5.5	0.189	0.0402
I/2	163.0	4.6	0.179	0.0344
II/2	160.0	5.5	0.176	0.0346
III/2	169.6+	6.3+	0.184+	0.0345+
IV/2	167.0	6.5	0.189	0.0350
Average:	163.3	5.5	0.181	0.0347
I/3	167.2	8.0	0.201	0.0422
II/3	164.0	6.9	0.196	0.0464
III/3	212.0+	14.5+	0.219+	0.0362+
IV/3	176.0	7.0	0.209	0.0470
Average:	169.1	7.3	0.202	0.0452
I/4	146.0	7.8	0.194	0.0452
II/4	156.0	7.0	0.197	0.0455
III/4	187.0+	11.0+	0.210+	0.0374+
IV/4	158.0	6.8	0.205	0.0468
Average:	153.3	7.2	0.199	0.0458
I/5	148.8	6.2	0.190	0.0446
II/5	141.6	5.7	0.194	0.0422
III/5	78.0+	3.7+	0.151+	0.0328+
IV/5	152.0	6.3	0.197	0.0460
Average:	147.5	6.1	0.194	0.0443
I/6	127.2	6.0	0.176	0.0446
II/6	133.6	5.6	0.187	0.0394
III/6	64.0+	4.8+	0.153+	0.0328+
IV/6	132.0	5.4	0.180	0.0400
Average:	130.9	5.7	0.181	0.0413
$S.D_{5\%}$	8.65	1.01	0.01	0.0034

According to both the relevant literature (MASSEY—LOEFFEL 1966, 1967) and our own data (GYŐRI—PALKOVICS 1974) the different parts of the maize grain have different zinc contents: for example, the germ + coleoptile has 96 ppm, the endosperm 30 and the pericarp 9. The accumulation of zinc in the different parts of maize shows the importance of the biochemical role of zinc. Here it should be noted that zinc is an important determinant of the quality of the crop, and that the tryptophane content changes parallel with the zinc content. The effect of increasing rates of phosphorus fertilization on the quality of the maize crop can be seen in Tables 1 and 2.

The data in Table 2 give evidence of a gradual decrease of the zinc content in the germ and endosperm alike, as a response to increasing rates of phosphorus application. The same trend was demonstrated for the tryptophane content.

Attention should be drawn to the fact that the highest zinc and tryptophane contents were observed in each case after the first application of phosphorus (Treatment 3). This can be explained by the low phosphorus level in the soil in the trial plots, which made the first dose of phosphorus indispensable for the normal development of maize. This first dose did not prevent the plants from taking up zinc from the soil. In this soil the application of nitrogen and potassium without phosphorus caused decided phosphorus deficiencies.

In studying the correlations between the increasing rate of phosphorus fertilization and the zinc and tryptophane contents the statistical method of variance analysis was used without taking the data of the two control plots (Treatment 0 and NK) into consideration, in order to decide whether the increasing fertilizer doses caused any considerable change. The extreme data marked with + in Table 2 were excluded from the evaluation.

Table 3 contains the significance levels of the variance analysis for all treatments in the trial and for the four phosphorus doses.

Table 3

Statistical tests of variance analysis for phosphorus fertilization

Parameters	F-test	
	treatment 1—6	treatment 3—6
Zn in germ	20.37***	36.33***
Zn in endosperm	6.76**	23.50***
Tr in germ	7.70**	12.44**
Tr in endosperm	15.10***	2.74
Grain weight	14.90***	1.81
Germ weight	12.34***	1.64
Germ %	6.18**	2.50
g/es ratio	7.34**	2.35

*** $P < 0.1\%$; ** $P < 1\%$; * $P < 5\%$.

A comparison of the statistical tests reveals that taking the 6 treatments in the trial into account reliable treatment effects can be observed for all parameters at least at the $P < < 0.01$ level but an increase in the rate of phosphorus application caused reliable changes only in the zinc contents of the germ and endosperm and the tryptophane content of the germ. The components of parameters showing significant treatment effects are broken down to their linear and quadratic components by means of orthogonal polynomials in Table 4.

Table 4

Variance table for correlation analysis calculated with orthogonal polynomials (Effect of phosphorus fertilization on the zinc and tryptophane contents of the grain components)

Factors	Zn in germ			Zn in endosperm			Tryptophane in germ		
	FG	MQ	F	FG	MQ	F	FG	MQ	F
Total	11			11			11		
Replication	2			2			2		
Treatment	3	744.59	36.33***	3	2.00	23.50***	3	0.000255	12.44**
Linear	1	2169.61	105.90***	1	5.46	64.23***	1	0.000694	33.85**
Quadratic	1	0.48	0.02	1	0.07	0.79	1	0.000065	3.18
Remainder	1	63.67	3.11	1	0.47	5.56+	1	0.000006	0.29
Error	6	20.49		6	0.08		6	0.000021	

*** $P < 0.1\%$; ** $P < 1\%$; * $P < 5\%$; + $P < 10\%$.

Regression equations and curves characteristic of the direction and extent of the change are presented in Fig. 1.

On the basis of the above the following facts can be established concerning the effect of phosphorus fertilization.

In the NK-treatment, and as a response to the lowest rate of phosphorus applied with a basic NK fertilization, the zinc content of the germ grew compared to the untreated plot, then with an equidistant increase in the phosphorus dose it linearly decreased at a 99.9% level of probability. According to the data of the variance table the linear effect of the negative regression is very intensive; the quadratic and residual components are not significant. The decrease produced by the application of the largest dose of phosphorus (280 kg)/ha P_2O_5) was 23% compared to the effect of the smallest one (70 kg)/ha P_2O_5). As to its absolute value (131 ppm), the zinc content in this treatment was even lower than that in the untreated plot (148.5 ppm). This statement agrees with the literary data, according to which high rate phosphorus fertilization causes a depression in the zinc uptake by the maize plant (Olson *et al.* 1962, Olson *et al.* 1965, Prohászka—Gurabi 1972, Prohászka—Cserni 1969).

Changes in the zinc content of the endosperm run parallel with those measured in the germ. In accordance with the regression equation in Fig. 1 the concentration of zinc is in linear correlation with the increase in the rate of fertilization. Under the influence of the largest dose of phosphorus the zinc content, which initially rose, fell to the level of the untreated plot. The extent of the change is not significant, but a correlation can be found between the accumulation of zinc in the two components of the maize grain.

As to the P—Zn antagonism different authors have different views. Langin *et al.* (1962) and Stukenholtz *et al.* (1966) explain the phenomenon as a physiological inhibition rather than a decrease in the readily soluble zinc content of the soil. Győri (1963) found no relationship between the mobile microelement content of the soil and the amount of microelement accumulated in the plants. Győri—Tölgyesi (1968) even found a negative correlation between the zinc contents of wild plants and the mobile zinc content of the soil, depending on the season. According to the investigations of Massey—Loeffel (1967) there was no correlation between the zinc deficiency of the soil and the zinc content of the maize grain.

The comparative evaluation of the data in the tables reveals a depressive effect of NK fertilization on the tryptophane content in the maize grain. Since this decrease is shown in both components of the maize grain (though with different degrees of reliablity), it may be

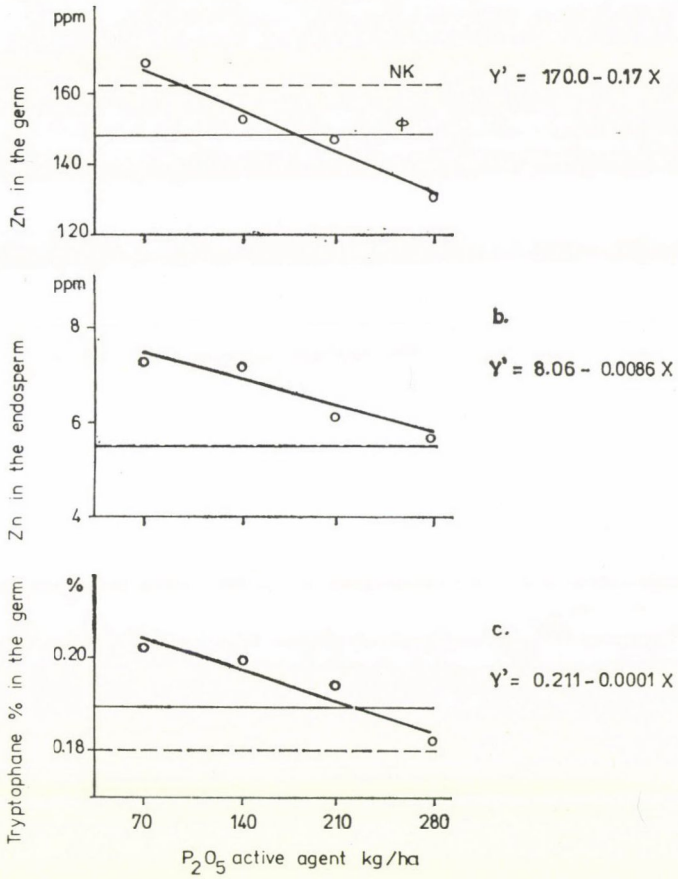

Fig. 1. Effect of phosphorus treatments on the zinc concentration of the germ and endosperm *(a, b)* and on the tryptophane concentration of the germ *(c)*

assumed that it is a phenomenon described by a number of authors (PRINCE 1954, NELSON 1956, VERESS 1973).

According to our own experience nitrogen fertilization resulted in a decrease in the tryptophane content of the maize grain in spite of the increasing protein percentage. This depressive effect was counterbalanced by the lowest rate of phosphorus application; in fact, the tryptophane content of the germ increased by 11% relative to the NK treatment. A similar effect was observed in the endosperm, with an increase of 30%.

With a further increase in the phosphorus dose the tryptophane content of the germ showed an approximately linear decrease. From the component of the regression treatment the linear effect at a $P < 0.01$ level was significant; the quadratic effect approached, but did not quite reach the $P < 0.1$ level of significance. On this basis the linear regression equation was calculated and presented in Fig. 1, where the placement of the points shows the effect of the quadratic components as well.

The largest dose of phosphorus reduced the value of tryptophane to the level of the NK basic treatment. The absolute value of the change is not high, but the trend calls attention

to a relationship between the extreme macro- and microelement effects and the grain components.

The regression of the tryptophane content in the endosperm as a response to phosphorus application was practically the same as in the germ; however, since the effect of the treatment was not reliable a regression equation was not calculated.

The F-tests of the variance analysis (Table 3) only prove that there is a reliable treatment effect on the weight conditions of the components when all the treatments are taken into consideration.

The germ weight and grain weight only changed in the NK and NKP_{70} treatments; higher rates of phosphorus application caused no further increase in weight. The g/es (g = = germ, es = endosperm) ratio showed an evenly rising tendency until the last treatment, but the changes only represent slight differences in value which do not reach the threshold of reliability. The correlation analysis was therefore made for those variables whose values changed through a wider interval under the influence of phosphorus fertilization. The data of series III, which was left out of the variance analysis, was included in the mathematical-statistical evaluation; the extremely high and low values of these data favourably widen the range of validity in the regression.

The values of correlation coefficients calculated from n = 8 data, together with the reliability percentages, are presented in Table 5. The regression equations and lines for significant correlations are shown in Fig. 2. The figure gives two equations obtained with different calculation methods for the germ zinc — germ tryptophane correlation; in one case the basis of calculation was formed by the averages calculated from series I, II and IV and the outstanding values of series III, and in the other case by the total data per plot.

Table 5

Correlation coeffixients (r) and their probability levels (P%) in a phosphorus fertilization trial

Correlation	r	P %
germ Zn : germ Tr	0.989	0.1
germ Zn : es* Tr	0.451	—
es Zn : es Tr	0.000	—
germ Zn : es Zn	0.834	1.0
germ Tr : es Tr	0.353	—

* Endosperm.

According to the data in Table 5 the closest correlation of all is found between the zinc and tryptophane contents of the germ, where the correlation coefficient r = 0.989 is significant at a $P < 0.001$ level. The correlation coefficient of germ Zn: germ Tr, calculated from n = 24 data using a frequency table, is r = 0.817, which is significant at a $P < 0.001$ level.

According to the result of the linearity analysis the effect of linear and non-linear components on the variance of tryptophane can be characterized as follows:

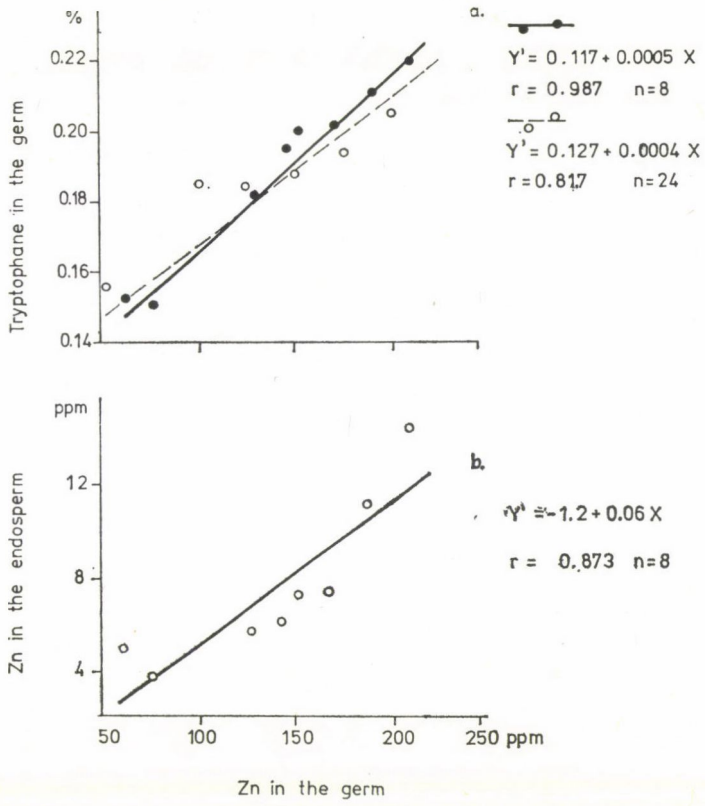

Fig. 2. Correlation between the zinc and tryptophane concentrations of maize grain in a phosphorus fertilization trial (*a* = germ Zn: germ tryptophane correlation; *b* = germ Zn: endosperm Zn correlation)

Component	Determinative coefficient, %
Linear component	66.76
Non-linear component	17.06
Other	16.18
Total	100.00

The highly significant r values obtained with the two different methods of evaluation, the equations shown in Fig. 2 and the regeression coefficients all prove realiably close correlation of zinc and tryptophane in the germ of the maize grain. There is a similarly close positive linear correlation, significant at a $P < 0.01$ level, between the zinc concentrations in the germ and endosperm.

The effect of phosphorus fertilization as seen in Fig. 1 again indicates a close correlation between the zinc and tryptophane contents in the germ and between the zinc contents of the germ and endosperm. The correlation coefficient for the zinc and tryptophane contents of the endosperm is not significant, which shows that the accumulation of tryptophane in the endosperm is not influenced by the small amount of zinc found in this part of the maize grain.

From the results of the component analysis a conclusion can be drawn on correlations valid for the whole grain. In the fertilization treatments the zinc content of the grain as a whole is determined primarily by the zinc content of the germ, as the relation of germ to endosperm changes only slightly under the influence of fertilization. The tryptophane content of the grain is influenced by the tryptophane concentration in both germ and endosperm.

As a response to fertilization the amino acid content in the two components changes in the same direction and to nearly the same extent. On this basis a correlation similar to that between the zinc and tryptophane content in the germ can be demonstrated for the whole grain. The result of the regression analysis can be summarized in a highly important rule, namely, that within the range of values concerned the tryptophane content increases linearly with the increase in the zinc content of the maize grain. A similar correlation was found by DIBROVA (1967) for the zinc nutrition of maize.

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Prepared at the Keszthely University of Agricultural Sciences, Department of Soil Science, Keszthely.

D. GYŐRI, G. MÁTZ

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FURTHER STUDIES FOR POTENTIATION OF CERTAIN ANTIFEEDANTS

The antifeedant properties of fentins for phytophagous insects have been investigated intensively on certain lepidopterous larvae, i.e. *Spodoptera littoralis*, *Agrotis ypsilon* (ASCHER—RONES 1964); *Heliothis* zea, *Heliothis virescens* and *Triptchoplusiani* (WOLFENBARGER *et al.* 1968); *S. littoralis* (RADWAN 1970); *Agrotis ypsilon* (YOUSEF 1974).

Reviewing the literature a lack of information could be observed concerning the joint action of antifeedants when combined with other groups. Recently, the joint action of antifeedants when combined with insecticides was studied and reported in two papers (ABO-ELGHAR *et al.* 1976 a, b) which represent the first part of this series. The potentiation of antifeedants due to combination with insecticides prompted us to study the potentiation of antifeedants when combined with each other. Thus, the results of tests with three antifeedants when combined in binary mixtures are reported in the present paper.

The technical grade of Du-Ter, Plictran and Brestan were used for studying the joint action of antifeedants in mixtures. Each antifeedant was tested either alone or in binary combinations with the others at ratios of 2 : 1, 1 : 1 and 1 : 2. A stock solution (w/v) of each material or mixture tested was prepared by dissolving the desired quantity of technical grade material in acetone. Subsequent serial dilutions (v/v) were freshly prepared before the test.

The 4th instar larvae of a laboratory strain of cotton leafworm *(Spodoptera littoralis)* were treated topically with the tested dosage on the dorsal surface of the third thoracic segment. For each dosage, four replicates of 10 larvae each were treated. The larvae were confined in 0.45 kg jars with 10 larvae/jar, and provided with castor bean leaves without any feeding marks or punctures. The leaves in the jars were replaced with fresh ones 24 hours after the treatment.

Mortality counts were recorded 24 and 48 hours after the treatment and were corrected according to ABBOTT's (1925) formula. The dosage-mortality lines were plotted on a logarithmic scale using the corrected mortality percentages. The co-toxicity coefficient based on the LD_{50} values was calculated adopting SUN—JOHANSON's (1960) method.

The consumed food area was estimated 24 and 48 hours after the treatment and subsequently the percentage protected area was calculated on the basis of the area consumed in the control. The percentage protected area was plotted against the dosage applied topically to the larvae on a probit/log dosage paper. Accordingly the PD_{90} (90% leaf-protection dosage) was calculated.

1. *The effect on toxicity.* The results in Table 1 demonstrate that some mixtures of different antifeedants have a synergistic effect on the cotton leafworm. A mixture of Plictran and Brestan has a highly synergistic effect on the cotton leafworm. The LD_{50} values for either Plictran or Brestan alone were 7.5 and 3.6 times higher, respectively, than that of their 1 : 1

mixture. This synergic effect could be attributed mainly to Plictran, as when the Plictran/Brestan ratio increased from 1 : 2 to 2 : 1, the LD_{50} values decreased from 7.6 to 1.1 μg/larva, while the co-toxicity coefficient increased considerably from 431.0 to 2998.0.

Mixtures of Plictran and Du-Ter also have a synergistic effect on cotton leafworm larvae but not as high as in the case of Plictran/Brestan mixtures. Results concerning Plictran/Du-Ter combinations indicated that all ratios except the 1 : 2 were synergistic after 24hr. The synergistic effect of Plictran was again confirmed, as when the Plictran/Du-Ter ratio increased from 1 : 2 to 2 : 1 the co-toxicity coefficient increased from 65.0 to 545.0; i.e. approximately 10 times.

Regarding the mixtures of Brestan/Du-Ter, it was evident that the combinations have a slightly synergistic effect. As the ratio of Brestan to Du-Ter increased, the LD_{50} values decreased, and consequently the co-toxicity coefficient increased from 83.2 to 275.0. The results obtained after 48 hr (Table 1) are generally of the same magnitude. It could be noted that all Plictran/Du-Ter ratios were synergistic and that the synergistic effect increased after 48 hr.

2. *The effect on protection percentage.* The effect of combining the tested antifeedants in mixtures on the rate of the consumed area and the protection percentages is presented in Table 2 as the dosage required to obtain 50 and 90% protection either 24 or 48 hr after treatment.

PD_{50} and PD_{90} values showed that combining Brestan and Plictran in one mixture has a synergistic effect on the protection percentage obtained, represented as a lowering of the dosages of the mixture required to obtain either 50 or 90% protection. A combination ratio

Table 1

The joint action of different antifeedants when combined and applied topically to 4th instar larvae of S. littoralis

Materials	Ratios applied	24 hr		48 hr	
		LD_{50}	Co-toxicity coefficient	LD_{50}	Co-toxicity coefficient
Plictran	1 : 0	45.00	—	40.00	—
Brestan	0 : 1	21.50	—	9.20	—
Plic. + Bres	2 : 1	1.10	2998.0	0.64	2959.0
	1 : 1	6.00	484.0	5.10	293.0
	1 : 2	7.60	341.0	4.60	269.0
Plictran	1 : 0	45.00	—	40.00	—
Du-Ter	0 : 1	105.00	—	54.00	—
Plic. + Du-T.	2 : 1	10.20	545.00	4.90	914.0
	1 : 1	50.00	126.00	10.40	442.0
	1 : 2	110.00	65.00	30.00	161.0
Brestan	1 : 0	21.50	—	9.20	—
Du-Ter	0 : 1	105.00	—	54.00	—
Bres. + Du-T.	2 : 1	10.65	275.0	10.10	127.0
	1 : 1	175.00	20.4	31.00	51.3
	1 : 2	55.00	83.2	24.00	85.2

Table 2

The effect of combining different antifeedants on the protecting potentiality when applied topically to 4th instar larvae of S. littoralis

Materials	Ratios applied	Protection % at			
		24 hr		48 hr	
		PD_{50}	PD_{90}	PD_{50}	PD_{90}
Brestan	1 : 0	1.950	10.30	0.880	10.20
Plictran	0 : 1	9.600	72.00	7.800	52.00
Bres. + Plic.	2 : 1	0.780	7.40	0.450	8.80
	1 : 1	0.920	10.00	1.150	10.00
	1 : 2	0.400	5.40	0.350	3.50
Brestan	1 : 0	1.950	10.300	0.880	10.20
Du-Ter	0 : 1	0.060	1.760	0.190	3.400
Bres. + Du-T.	2 : 1	0.014	0.380	0.040	0.500
	1 : 1	0.072	10.00	0.185	2.400
	1 : 2	0.036	0.760	0.014	0.310
Du-Ter	1 : 0	0.060	1.70	0.190	3.40
Plictran	0 : 1	9.600	72.00	7.800	52.00
Du-T. + Plic.	2 : 1	0.015	1.30	0.054	1.05
	1 : 1	0.010	1.10	0.065	1.55
	1 : 2	0.050	0.86	0.025	0.50

of 1 : 2 (Brestan/Plictran) was the most promising, indicating 50 and 90% protection of the area consumed at 0.4 and 5.4 μg/larva in comparison to 1.95 and 10.3 μg/larva for Brestan and 9.6 and 72.1 μg/larva for Plictran to obtain 50 and 90% protection, respectively, after 24 hr. It could be noted that the results obtained after 48 hr showed almost the same trend.

As for combinations of Brestan and Du-Ter, the results obtained indicated that while a Brestan/Du-Ter ratio of 2 : 1 was the most promising after 24 hr, a 1 : 2 ratio of the compounds was the most promising after 48 hr.

Regarding Du-Ter/Plictran combinations, it is obvious that a 1 : 2 ratio of Du-Ter/ /Plictran was the most promising in increasing the protection percentage, where the dosages required for 90% protection were 0.86 and 0.50 μg/larva in comparison to 1.7 and 3.4 μg/larva for Du-Ter and 72.0 and 52.0 μg/larva for Plictran after 24 and 48 hr, respectively.

In general, it could be concluded that combining certain antifeedants increased the potentiality of such compounds in protecting foliage. These findings were in agreement with toxicity results (Tables 1) and confirm that there is a positive correlation between the increase in toxicity and the protection obtained and that the antifeeding effect referred mainly to the toxicity of the compounds, as previously recorded by RADWAN (1970).

*

Prepared at the Department of Plant Protection, Faculty of Agriculture, Tanta University, Shebin El-Kom.

M. R. ABO-ELGHAR, H. S. A. RADWAN, A. I. FARAG.

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IN MEMORIAM PROF. GY. MÁNDY

BIOLOGICAL ACTIVITY OF 6-METHYLURACIL (6-MU) IN COMPARISON WITH PESTICIDE-TYPE DERIVATIVES

Large-scale technology in agriculture requires pesticide (biological active compounds) applications covering herbicides, insecticides and fungicides. In recent years very intensive research for chemical factors has been initiated in studying new types of biological active compounds, since it has become clear, on the basis of earlier experience, that the derivatives of basis compounds possess a special, though very varying effectivity in almost every case. This can be used to advantage in chemical processing in agriculture. This principle has been demonstrated from several aspects in derivatives of basic compounds: phenoxyacetic acid, carbamates, dithiocarbamates, etc. (JUNG 1972, BÁNKI 1976).

The present study deals with the biological efficiency of 6-MU as a basic compound, related primarily to chemical processing in agriculture, thus correlations are given between the chemical structure of the derivatives and their special biological activity. The biological activity of 6-MU derivatives was discussed in an earlier paper (POZSÁR—MATOLCSY 1968). It was then found that at the ppm level this compound stimulates the intensity of protein synthesis and nucleic acid synthesis during very short expositions, and characteristically inhibits the breakdown of chlorophyll. It also possesses morphogenetic effectivity. On the basis of all these, the biological activity of 6-MU (pseudothymine) has been qualified as that of a hormone-like compound, constituting a transition between uracil and thymine. On the other hand, it was found indirectly that the potential precursors of 6-MU (the sodium salt of beta-uramine crotonic acid and the ethylester of beta-uramine crotonic acid) had a biological effectivity similar to the basic compound (MATOLCSY—POZSÁR 1969) in the stimulation of the intensity of nucleic acid synthesis. By inference from this, the opinion was held that the two precursors with open carbon chains are able to form a ring closure reaction in plant tissues.

The effect of 6-MU and some of its derivatives has been studied in detail for dry matter increment in the mycelium culture of higher basidial fungi (*Agaricus bisporus* Möll. et Schäff.),

for the accumulation of different nitrogen compounds, and for increasing the protein-nitrogen ratio, in comparison with the efficiency of several other hormone-like compounds, and of bioactive factors. The experimental results relating to mycelium cultures of *A. bisporus* and to *Coprinus comatus* Fr. were reported in detail (SZABÓ *et al.* 1972). In the present study only the effectivity of 6-MU and its derivatives in increasing the protein level has been emphasized, this being one of the characteristics of the biological activity in the basic compund, or in another interpretation, a significant side-effect of the compound from a biological point of view. The effectivity of 6-MU with respect to the intensity of protein synthesis has also been studied with the isotopic tracer technique.

The effect of potential pyrimidine base analogues on the intensity of nucleic acid synthesis has been classified with a comparative method, leaf disc bioassay. The method has been described in an earlier publication (POZSÁR—MATOLCSY 1969). The experimental data may explain the effect of the aza-derivatives introduced into the fifth and sixth positions, in comparison with the effect of 6-MU on nucleic acid synthesis.

The experimental results published were used to analyse whether divergent trends could be distinguished in the protected cultivated plants and in the leaves of sensitive and resistant weeds with respect to the effect of the 6-MU derivatives Isocil and Venzar on the nucleic acid synthesis. An attempt has been made to characterize the ralative biological stability of 6-MU as a basic compound. In this respect the methyl group was labelled with radioactive carbon ($—^{14}CH_3$), and the radioactivity of the basic compound was determined in Pinto bean leaf discs, at different intervals after the separation. Finally, an attempt was made to evaluate the long-term effectivity of 6-MU treatment with the aid of relative quantities of different protein fractions. On the basis of different molecular weights the fraction of protein produced a direct demonstration for the evaluation of the biological effectivity of 6-MU in the dynamic conversion at the protein level.

In the discussion a detailed analysis will be given to explain the biological effectivity of bioactive 6-MU as a basic compund and of three different groups of its derivatives by exploring the correlations between chemical structure and biological effectivity.

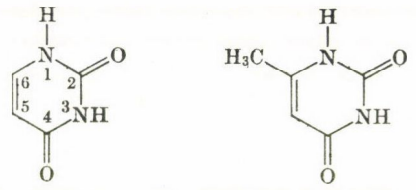
The tracer technique used in leaf disc tests was described in detail in earlier publications (POZSÁR—MATOLCSY 1968, 1969; MATOLCSY—POZSÁR 1969). In general Pinto bean leaves were used in testing, but in some cases Xanthi-nc tobacco leaves of triploid sugar beet ("Sopronhorpács" variety), *Sinapis arvense* L., *Viola tricolor* L. and *Reseda lutea* L. were also used. *S. arvense* represented the weeds sensitive to herbicides, and *V. tricolor* and *R. lutea* those resistant in a comparative series of experiments. The effect of the bioactive compounds was generally evaluated in a short exposition, so the leaf discs were floated on solutions of different bioactive compounds in ppm concentrations for 18 hours, then placed on solutions of labelled amino acids, pyrimidines or purines for 3 hours. After incorporation and the repeated purification of the fractions their radioactivity was measured by the liquid scintillation method.

The protein synthesis and the total nitrogen content in mycelium cultures under the influence of several hormone-like compounds and other bioactive factors were examined according to our publications (SZABÓ *et al.* 1972). The different protein fractions were coagulated by means of 10% trichloroacetic acid after repeated purifications, while the nitrogen content of the different fractions were determined by means of the micro-Kjeldahl method.

The chlorophyll retention test and the method of chlorophyll determination were described in detail in our earlier studies (POZSÁR—MATOLCSY 1968, POZSÁR 1971).

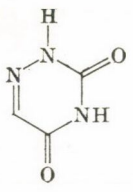
The structural formulae of bioactive 6-MU and related compounds are shown in Fig. 1. Besides the asymmetric and symmetric aza-uracils, the effect of several pyrimidine analogues and potential pyrimidine analogues has also been compared by means of radioactivity, with the tracer method, tested by leaf disc bioassay, which was described in an earlier publication (POZSÁR—MATOLCSY 1969).

The relative biological stability of 6-MU in the leaf tissues was compared in short and long expositions with the aid of radioactive 6-MU, labelled with ^{14}C in the methyl group. 6-MU with identical radioactivity was placed on the leaves of Pinto bean, and separated after 0, 1, 7 and 14 days; the radioactivity was determined by means of the liquid scintillation method. The effectivity of the 6-MU treatment in a long exposition was investigated on the basis of the molecular weight fractionation of protein, according to Table 1. The fractionation of leaf protein by means of Sephadex-150 gel chromatography was described in detail in another publication (POZSÁR 1972).

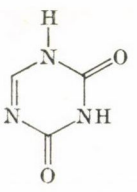


Uracil

6-Methyluracil 6-MU
(Pseudothymine)



6-Azauracil



5-Azauracil

Fig 1. Structural formulae of bioactive 6-MU and related compounds

Table 1

Effect of benzyladenine, benzimidazole and 6-MU on the intensity of protein synthesis in Pinto bean leaf discs, after floating on bioactive solution for 18 hours, then testing the incorporation of radioactive carbon-labelled glycine-2-^{14}C (with 26 mCi/mmol specific activity) from an external solution of 50 ml volume and 0.5 μCi/ml activity during 3 hours' exposition, related to cpm/200 mg fresh weight and as a percentage of the control

Treatment	ppm	Radioactivity 1000× cpm/200 mg fresh weight	Stimulation as a percentage related to the control
Distilled water	—	45.5	—
Benzyladenine	30	85.1	87.0
Benzimidazole	200	63.5	37.3
6-MU	100	67.0	47.2

Surveying the data of our earlier studies (Table 1) the stimulation influence of 6-MU on the intensity of protein synthesis can be seen in comparison with the stimulation induced by benzyladenine and benzimidazole. The biological effectivity examined in a short exposition is very conspicuous, as is the intensity of protein synthesis, which could be characterized by glycine-2-^{14}C incorporation, and which increased to 47% under the effect of 6-MU and to 87% under the influence of synthetic cytokinin. (It is worth mentioning that the stimulation due to benzimidazole and other imidazole type systemic fungicides was 37%.) In the earlier study it was concluded that the effectivity of bioactive compounds could be examined by the intensity of nucleic acid synthesis (POZSÁR—MATOLCSY 1968), followed as the rate of the incorporation of labelled nucleobases.

Examining the biological activity of 6-MU by means of several biotests, we succeeded in establishing that under the influence of long-term treatments the chlorophyll contents and the chlorophyll preservation increase (POZSÁR—MATOLCSY 1968). In leaves or half-leaves treated with 6-MU the breakdown of chlorophyll was inhibited due to the influence of the bioactive compound when the control was floated on water. During long-term treatments 6-MU in 100 ppm concentration increased the chlorophyll content of Pinto bean leaves to 47%, related to untreated intact leaves of half-leaves. The effect on the increase in the chlorophyll content is also very conspicuous, because it considerably exceeds the stimulation induced by benzimidazole (to 40%) and benzyladenine (to 41%) (Table 2).

Table 2

Effect of 6-MU, benzimidazole (BI) and benzyladenine (BA) on the stimulation of chlorophyll contents in the leaves of Pinto beans during long-term treatments. The half-leaves were treated with bioactive solutions daily for a week, while the control half-leaves were treated with water; during the second week after a period without treatment the chlorophyll determination was performed on the 14th day, expressed in extinction (660 nm), related to 200 mg fresh weight, and in comparison with the intact half-leaf control as a percentage

Tests	Water-treated half-leaves	Bioactive compounds			Stimulation of the chlorophyll contents as a percentage of the control half-leaves
		6-MU	BI	BA	
Intact leaf	1.08	—	—	—	—
Half-leaves	0.95	1.40	—	—	47.3
Half-leaves	0.90	—	1.26	—	40.0
Half-leaves	1.10	—	—	1.50	41.8

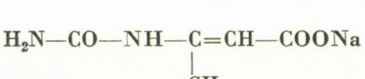
The chlorophyll contents and the inhibition of the breakdown of chlorophyll may be in positive correlation with the relatively high endogenous cytokinin level, and with its effect in increasing the soluble protein fractions (POZSÁR 1971).

The effect of potential precursors of 6-MU on the intensity of ribonucleic acid synthesis in Xanthi-nc tobacco leaf discs is shown in Table 3 on the basis of our published results (MATOLCSY—POZSÁR 1969). 6-MU stimulated the ribonucleic acid synthesis, characterized by the 50% incorporation of radiocarbon-labelled uracil, while its potential precursors, the sodium salt of betauramine crotonic acid and the ethylester of beta-uramine crotonic acid gave 21% and 23% incorporation respectively. The short exposition tests point to the fact that the possibility of 6-MU formation by ring closure does exist, in spite of the fact that this has not been directly verified.

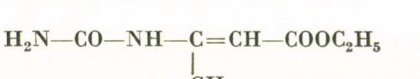
Table 3

Effects of the sodium salt of beta-uramine crotonic acid, and the ethylester of beta-uramine crotonic acid, as well as 6-MU and uracil tested by means of the incorporation of radiocarbon-labelled uracil-2-^{14}C, with 3.7 μCi/mmol specific activity, into the ribonucleic acid in Xanthi-nc tobacco leaf discs. After 18 hours of floating on the bioactive solutions with 100 ppm concentration the dics were transferred to 50 ml labelled nucleobase solution, with 0.5 mCi/ml radioactivity, for 3 hours. The radioactivity was expressed in 1000 cpm related to 100 mg fresh weight, while the specific activity was calculated to the protein mg, and a comparison was also made using percentage values of the stimulation referred to protein mg

Bioactive compounds	Radioactivity 1000 cpm/100 mg fresh weight	Mean error of mean value	Specific activity, cpm/mg	Stimulation protein as a percentage
Sodium salt of beta-uramine crotonic acid	357.2	26.4	370.5	21
Ethyl ester of beta-uramine crotonic acid	342.8	23.8	376.6	23
6-MU	416.4	37.8	475.5	50
Uracil	276.6	21.5	303.9	—



sodium salt of beta-uramine crotonic acid



ethylester of beta-uramine crotonic acid

6-MU and 2-thio-6-methyluracil considerably stimulated the dry matter contents in the mycelium cultures of tested basidial fungi over 21 day periods, in all three concentrations examined (1, 10 and 100 ppm). According to the experimental data shown in Table 4, the effect of a 1 ppm concentration is much more favourable than the influence of 10 ppm and in both pyrimidine bases the highest concentration induced the most unfavourable stimulating effect. Comparing the effects of the many hormone-like compounds and bioactive factors,

Table 4

Effect of 6-MU and 2-thio-6-methyluracil on the increment of dry material in the mycelium culture of A. bisporus and of C. comatus during a 21 day incubation period, expressed as a percentage of the control

Bioactive compounds	ppm	Dry matter in mg		Stimulation as a percentage	
		Agaricus	Coprinus	Agaricus	Coprinus
Control	—	7.17	6.69	100	100
6-MU	1	8.79	13.76	122	205
6-MU	10	10.02	12.00	139	179
6-MU	100	13.34	11.16	186	166
2-Thio-6-MU	1	15.27	13.91	213	208
2-Thio-6-MU	10	13.29	12.28	185	183
2-Thio-6-MU	100	9.58	10.87	133	162

Table 5

Stimulating effect of 2-thio-6-methyluracil on the increase in the total nitrogen (crude protein) quantity related to the dry matter contents in A. bisporus mycelium cultures, at 100 ppm concentration, during a 21 day incubation period

Bioactive compound	Dry matter in mg	Total nitrogen content as a percentage of dry matter	Stimulation as a percentage of the control
Control	7.17	4.35	—
2-Thio-6-MU	9.58	7.73	78

the stimulating effectivity of 6-MU and its 2-thio derivative can be considered very important (SZABÓ *et al.* 1972) as far as organic matter is concerned. Table 5 shows the rate of the stimulating effect of 2-thio-5-methyluracil induced in a 100 ppm concentration on the *A. bisporus* culture, according to an earlier study (SZABÓ *et. al.* 1972). The total nitrogen content increased by 78% during a 21 day treatment period under the influence of the bioactive compound, while the basic compound did not manifest this stimulating character. The experimental results shown in Table 6 are also of outstanding importance in connection with the increase of the relative quantity of protein-nitrogen content, related to the total nitrogen level. The ratio of protein-nitrogen in the total nitrogen content considerably increases under the influence of the two pyrimidine base analogues. According to the data in the earlier study referred to above (SZABÓ *et al.* 1972) the protein-nitrogen ratio was increased by 7% with 6-MU, and by 3% with 2-thio-6-methyluracil in the highest concentration applied. It should be taken into consideration that 6-MU exerts a characteristic influence on the increment in dry matter contents, while its 2-thio derivative affects the total nitrogen content and also the increase in the protein-nitrogen ratio in the case of mycelium cultures. Our aim was to determine whether 6-MU also affected the intensity of protein synthesis in the mycelium culture of *A. bisporus* and not only the protein-nitrogen content or its ratio in the total nitrogen level. This question is answered in the affirmative in Table 7, as 6-MU increased the intensity of protein synthesis by 59% compared to thymine and uracil, when testing the glycine-2-^{14}C incorporation in 3 hours after a short 18 hour incubation period. Table 7 offers comparative data related to the two nucleic bases, and the conclusion may be drawn that its effect may be exerted not through conversion by demethylation or transmethylation as a natural nucleic basis, primarily the guanine in

Table 6

The influence of 6-MU and 2-thio-6-methyluracil on the increase in the protein-nitrogen ratio related to the total nitrogen content in a mycelium culture of A. bisporus during a 21 day incubation period

Bioactive compounds	ppm	Protein-nitrogen in the total nitrogen content, %	Stimulation as a percentage of the control
Control	—	64.3	—
6-MU	10	65.3	1.6
6-MU	100	68.9	7.0
2-Thio-6-MU	100	66.4	3.2

the 9 (methyl) and 6 (methoxy) positions, but as a relatively stable uracil derivative, which is a consequence of the bioactivity.

Comparative studies were carried out with several potential pyrimidine base analogues, by testing their biological effectivity with the aid of the intensity of ribonucleic acid synthesis. These studies were described in earlier publications (POZSÁR—MATOLCSY 1968, 1969). According to the experimental results presented in Table 8, uracil inhibited the incorporation of radioactive uracil, compared to the untreated control, and this phenomenon can be explained by the relatively greater uracil concentration in the plant tissues. On the other hand, 6-MU stimulated the intensity of ribonucleic acid synthesis by 22%, and 5-azauracil by 17% in Pinto

Table 7

The effect of 6-MU on the intensity of protein synthesis was characterized with the incorporation of radioactive carbon-labelled glycine-2-^{14}C with 26 mCi/mmol specific activity in a mycelium culture of A. bisporus, compared to the effect of thymine and uracil. The bioactive compound and the natural nucleic bases were used in 100 ppm concentration, and were tested after an 18 hour incubation period with the 3 hour tracer method. The radioactivity was expressed in 1000 cpm/mg dry matter, and the stimulation as a percentage of the control

Pyrimidine bases	Radioactivity 1000 cpm/mg related to the dry matter	Mean error of mean value	Stimulation as a percentage of the control
Water	13.2	0.83	—
Thymine	13.1	0.72	—
Uracil	13.4	0.96	2
6-MU	21.4	1.70	59

Table 8

The effect of 6-MU on the intensity of ribonucleic acid synthesis was characterized by the incorporation of radioactive carbon-labelled uracil-2-^{14}C, with 3.7 mCi/mmol specific activity, compared to the effectivity of uracil, 5-azauracil and 6-azauracil, in the leaves of Pinto bean, after 18 hours floating on the surface of bioactive solutions at 100 ppm. After a 3 hour exposition period the radioactivity at the labelled nucleobase was expressed in 1000 cpm/100 mg fresh weight and the rate of deviation from the control as a percentage

Pyrimidine base analogues	Radioactivity 1000 cmp/100 mg fresh weight	Difference as a percentage of the control
Water	74.4	—
Uracil	58.7	78
6-MU	91.5	122
5-Azauracil	87.2	117
6-Azauracil	40.8	54

bean leaves, in an analogous manner. 6-azauracil, as an asymmetric analogue, displayed a 54% effectivity compared to the control. The phenomenon explains the influence of 6-MU exerted on both protein synthesis and protein level through ribonucleic acid synthesis. In this connection an explanation can be given for the regularities in the changes of bioactive character correlated with the chemical structure. Comparing the biological activity to the uracil effect according to the data in Table 8, the stimulation of ribonucleic acid synthesis induced by 6-MU increased by 55%, and the effectivity of 5-azauracil by 48%. This difference is of a very remarkable extent and is connected with the analogy in the chemical structure, considering the 18 hour short treatment periods.

Recognizing that 6-MU exerts its bioactivity through ribonucleic acid synthesis, our investigations were extended to two 6-MU derivatives of herbicide character, namely Isocil and Venzar, marketed by Du Pont. It was assumed that the pyrimidine type herbicides with 6-MU basic characters did not influence the ribonucleic acid metabolism of resistant culture plants, but only that of sensitive weeds. On the basis of this supposition, the effects of two herbicides on the ribonucleic acid synthesis were examined in culture plants, and in sensitive and resistant weeds, in order to interpret the action mechanism (Table 9). The herbicide activity of 6-MU derivatives only inhibited the ribonucleic acid synthesis of sensitive weed leaves. No change could be detected in the ribonucleic acid synthesis of either protected culture plants or resistant weed leaves due to this effect. In this case an analogous biochemical action mechanism was demonstrated.

Table 9

Effect of 6-MU type herbicides (Isocil, Venzar) on the ribonucleic acid synthesis in the leaf discs of a culture plant (sugar beet, "Sopronhorpács" triploid variety) and of susceptible (S. arvense L.) and resistant (V. tricolor L. and R. lutea L.) weed plants tested by the incorporation of radiocarbon-labelled uracil-2-^{14}C, with 3.7 mCi/mmol specific activity, after an 18 hour herbicide treatment at 100 ppm concentration of active material, and after a 3 hour exposition on the labelled nucleobase solution. The radioactivity was expressed in 1000 cpm/mg fresh weight, and the rate of deviation from the control as a percentage

Test plants	Herbicides in the treatments	Radioactivity 1000 cpm/ g fresh weight	Mean error of the mean value	Difference as a percentage of the control
Sugar beet	Control	86.7	5.23	100
Sugar beet	Isocil	81.5	6.71	94
Sugar beet	Venzar	88.2	5.80	101
S. arvense	Control	112.6	8.64	100
S. arvense	Isocil	86.9	5.81	77
S. arvense	Venzar	72.4	6.13	64
V. tricolor	Control	59.0	3.92	100
V. tricolor	Isocil	62.1	5.04	105
V. tricolor	Venzar	57.4	4.17	97
R. lutea	Control	94.2	6.30	100
R. lutea	Isocil	96.8	7.12	102
R. lutea	Venzar	93.5	5.93	99

Subsequently the relative biological stability of the bioactive basic compounds was examined in the tissues of Pinto bean leaves. Identical quantities of the radiocarbon-labelled basic compound were placed on the intact leaves. From samples taken immediately after the application (0 day), then on the following day (1st day), and under the conditions of a long-term treatment at the end of the first week (7th day) and the end of the second week (14th day), the radioactivity of 6-MU was determined after fractionation and radiochemical identification as demonstrated in Table 10. After the first day only 15% of the labelled compound decomposed, while up to the end of the first and second weeks 28 and 33% were destroyed. It thus seems that 6-MU is relatively stable in the plant tissues, so its quantity does not decrease with the demethylation processes. The 6-methyl group of the other 6-MU derivatives are relatively stable biologically.

Table 10

Relative stability of 6-MU, with 14.6 mCi/mmol specific activity, labelled with radiocarbon on the methyl group, in the leaves of Pinto bean, with the aid of separations after different exposition periods (0, 1, 7, 14 days). The radioactivity was expressed in 1000 cpm/μg, with the mean error of the mean values, and the relative stability as a percentage of the original activity of the model compounds

Days of separation	Radioactivity in 1000 cpm/μg	Mean error of the mean value	Difference as a percentage of the original activity
0	54.7	6.41	100
1	46.5	5.91	85
7	39.4	4.23	72
14	36.7	4.06	67

Finally, a detailed investigation was carried out to fractionate the protein by molecular weight in juvenile and senescent leaves and in healthy and fungi-infected ones, and these results were compared with the effect of several bioactive compounds. Table 11 shows the effect of 6-MU on the shift in the ratios of different protein fractions in Pinto bean leaves.

Table 11

Effect of 6-MU treatment in 200 ppm concentration, daily for one week, then after the subsequent resting period at the end of the second week, on the change in the protein fractions with different molecular weights in the leaves of Pinto bean. The protein content was 21.1%, related to the dry matter

Molecular weight (1000×) of protein fractions	Control	6-MU	Difference as a percentage of the control
12	3.2	4.2	131
24	4.0	4.8	120
36	5.1	5.1	100
120	6.2	5.7	91
400	2.6	1.3	50

Under the influence of the long term treatment, the ratio of the low molecular weight protein fractions increased (the 12 000 and 24 000 fractions by 31 and 20%, respectively), the middle fractions remained unchanged and the high molecular weight fractions (120 000, 400 000) decreased by 9 and 50%, respectively. According to the data, the increase in the ratio of fractions with low molecular weights (soluble fractions), and the simultaneous decrease in the quantities of the fractions with high molecular weights, are in an analogous relationship with the effectivity of cytokinins and systemic fungicides. An opposite or reversed process in the conversion of juvenility into senescence, or the development of pathological processes to partial necrosis.

On the basis of the experimental results discussed above, it can be inferred that 6-MU has a remarkably high biological effectivity on protein synthesis, protein level, and the relative growth ratio of low molecular weight protein fractions. The action mechanism of 6-MU on the protein metabolism in the leaves can be considered as indirect, since primary stimulation can be directly demonstrated at the level of ribonucleic acid synthesis. On the basis of the biological activity exerted by this compound on ribonucleic acid synthesis, it can be inferred that the influence of 6-MU may also have a stimulating effect on the deoxyribonucleic acid synthesis. The relative stability of the bioactive basic compound was observed in the leaf tissues and the original level did not decrease to 33% even during a two-week long-term treatment.

It may be of interest to compare the effectivity of 6-MU in influencing ribonucleic acid synthesis with that of 5-azauracil, 6-azauracil or other pyrimidine derivatives.

A comparative treatment could be made in principle in terms of the lone-pair, or π-electron ionization potentials of nucleic acid bases. This treatment of the problem, however, would involve these bases taking part in some way in the formation of charge-transfer (or any other related) complexes. Recently such approximations have frequently been made in connection with a number of important problems in biochemistry. On the basis of quantum biochemical calculations PULLMAN—ROSSI (1964) have concluded that when nucleic acid bases are investigated, the lowest ionization potential corresponds to the ionization of a π-electron and for all bases the order of increasing ionization potential is:

$$\pi < n(0) < n(N) .$$

It is of interest to mention that uracil appeared to be both the worst π-donor and the worst lone pair: n-donor.

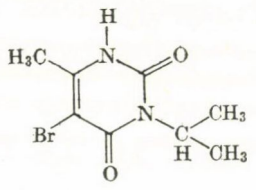
Although correct ionization potentials are not available either for 5-azauracil, 6-azauracil or 6-MU, it should be emphasized that on the basis of the electron donor capacities of nucleobases 6-MU as well as 5-, and 6-azauracil should be biologically more active than uracil. This has been found earlier and in the present work as well.

Nevertheless, the assumption of any correlation between the biological effectivity and the lone-pair or π-electron donor ability of nucleobases appears to be an over-simplification, because this would suggest that pyrimidines with higher π-electron densities or substituted with electron-repelling substituents should exhibit higher biological activity. Such a correlation has not yet been reported.

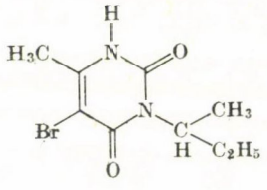
The 5-azauracil effect can be explained from the analogous biological characteristic of 6-MU, which displays an identical action mechanism in the stimulation of both protein and ribonucleic acid synthesis. On the other hand 6-azauracil, with an asymmetric structure, induces a smaller and more unfavourable biological effectivity, despite its sixth position.

6-MU has not been included in pesticides, despite the synthesis described by DONLEAVY—KISE (1963). DEKKER—OORT (1964) on the other hand raised the question of the fungicide effectivity of 6-azauracil against powdery mildew. The possibility of using bases of 6-MU structure was not included in the literature until 1968, however. AUDUS (1964) and

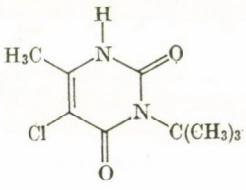
TERÉNYI *et al.* (1967) did not categorize pesticides and herbicides of the pyrimidine type. The first manual in which a relatively large number of pesticides with 6-MU structure are presented is that edited by MARTIN (1971), though the list is not complete, and objections could be raised to several structural formulas. The 6-MU type pesticides patented and marketed until recently, and listed on the basis of the mentioned manual, and of relevant catalogues, can be classified according to the categories presented below. Figure 2 shows the 6-MU derivatives of herbicide character. It is generally characteristic of these that they occur as imino nitrogen, and contain hydrogen in the first position, so the formation of the riboside bond is potentially possible, as proved unequivocally by several authors, on the basis of experimental results obtained by us and by other researchers (GRUNBERG—MANAGO—MICHELSON 1964, FOX *et al.* 1966, ŠKODA *et al.* 1966). In positions 2 and 4 there are free oxo-groups for securing hydrogen bonds, which make it positionally possible for the bioactive base to link with the macromolecules. The 6-MU structure guarantees a biological effectivity that also appears not infrequently as a side-effect in the case of derivatives, and determines the preservation of the speciality of 6-MU as a free base (occasionally in the form of nucleoside or nucleotide), unlike uracil, which is necessarily used for ribonucleic acid synthesis. Special bioactivity



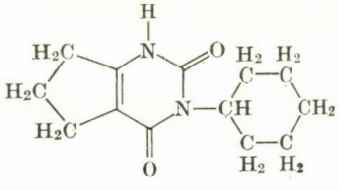
1. Isocil



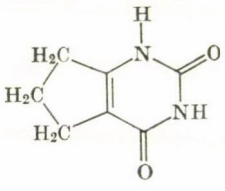
2. Bromacil



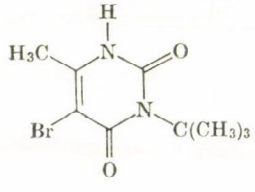
3. Sinbar



4. Venzar



5. Lenacil



6. Du Pont 733

Fig. 2. 6-MU derivatives with herbicide character

is also ensured for compounds of 6-MU structure by the fact that their demethylation is very slow, even under oxidative conditions (MANZER 1973, EDWAHRS 1974, KEITH 1976, JOLLEY *et al.* 1976). Without exception 6-MU derivatives with herbicide effectivity contain exclusively hydrocarbon (saturated aliphatic) groups in positions 3, 5 and 6, with relatively small variation.

It can be considered very probable that herbicides with the structure described in detail below appear primarily and exclusively as uracil analogues. The reactivity of the side chains is minimal; they inhibit translation and transcription in susceptible organisms largely due to a steric effect.

1. Isocil, Hyvar, Du Pont product, 3-isopropyl-5-brome-6-methyluracil.
2. Bromacil, Hyvar X, Du Pont product, 3-secunder,butyl-5-brome-6-methyluracil.
3. Sinbar, Du Pont 732, Du Pont product, 5-chloro-3-tercier,butyl-6-methyluracil.
4. Venzar, Du Pont product, 3-cyclohexyl-5,6-trimethyl-uracil.
5. Lenacil, Du Pont 634, Du Pont product, 5,6-trimethenyl-uracil.
6. Du Pont 733, Du Pont product, 5-brome-3-tercier, butyl-6-methyluracil.

Figure 3 demonstrates superselective fungicides which are derivatives of 6-MU (8 and 9), insecticides which are compounds of 6-MU structure (7 and 10), and a special rodenticide (11). The chemical structure of the compounds can be characterized as follows:

7. Pirimiphos-methyl, PP 511, ICI product, 2-diethyl-amino-6-methylpyrimidine-4-yl-dimethyl-phosphorothional.
8. Dimethrimol, BSI product, 5-n-butyl-2-dimethyl-amino-4-hydroxy-6-methylpyrimidine.
9. Ethirimol, ICI product, 5-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine.
10. Pirimicarb, PP 062, ICI product, 2-dimethyl-amino-5,6-dimethylpyrimidine-4-yl-dimethylcarbamate.
11. Crimidine, ISO, Castrix, Farbenfabrik Bayer AG product, 2-chloro-4-dimethylamino-6-methylpyrimidine.

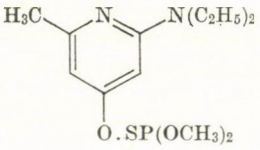
It is characteristic of selective fungicides with a 6-MU structure (8 and 9) that the nitrogen in the first position is of imido character, but potentially it is capable of bonding the nucleoside formation by means of a hydrogen bridge. The hydroxyl bonding to the fourth position can be directly involved in hydrogen bonds, so its association with macromolecules is probable. The N-methyl, N-ethyl, and N-butyl groups bonding with the side-chains change very rapidly and easily, in contrast with the C-methyl (C-alkyl) groups, which can be considered to be very stable.

The nitrogen in the first position of 6-MU structured insecticides has an imino character, but it is free, and potentially free to produce nucleoside and nucleotide type compounds very easily. The hydroxyl or oxo groups only occur potentially in the fourth position. In the case of pirimiphos-methyl the formation of free hydroxyl is very probable, and the dissolubility of the compound offers a possibility for the association with macromolecules, but only together with a decomposition decrease in biological activity, and the transfer of free methoxy groups. Owing to the transfer of the methoxy groups, the formation of sulphur and phosphorus ester also offers reactive conditions in the cell region. The metabolism of Pirimicarb can be regarded as following a much simpler course, owing to the demethylation, deamination and decarboxylation of the amino groups formed in the meantime. The rodenticide character of the 6-MU compound is very different from the uracil character; it is only the imino in the first position and the structure of the ring that is identical, the effecting side-groups do not recall base pairing, even after further metabolic transformations.

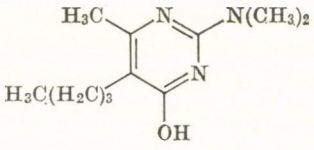
These compounds (7—11) do not contain hydrogen atoms in the first position, and hydrogen bonding with different macromolecules and with riboside (deoxyriboside) is impos-

sible (VINOGRADOV—LINNELL 1971, LADIK 1972), even at the enzyme level (FROMM 1975). The presence and absence of hydrogen in the first position shows a possibility of bioenergetical differences between the biological activity of the first (1—6) and second (7—11) groups of uracil derivatives.

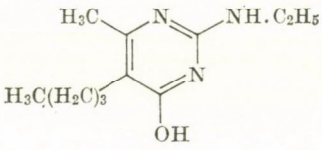
6-MU derivatives have recently been used in Hungary in experimental weed control and in agricultural practice. On the basis of the manual by UBRIZSY—GIMESI (1969) they have already been included in the relevant plant protection literature.



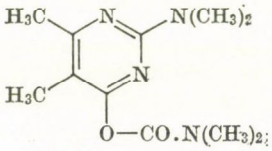
7. Pirimiphos-methyl



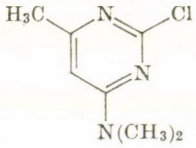
8. Dimethrimol



9. Ethirimol



10. Pirimicarb



11. Crimidin

Fig. 3. Superselective fungicides (8, 9), insecticides (7, 10), and a special rodenticide (11)

On the other hand, the rather general biological effectivity of pyrimidine derivatives can probably be explained by assuming that pyrimidines as general proton donors and/or proton acceptors can influence the secondary (or tertiary) structure of proteins and also of nucleic acids. No direct chemical evidence supports this suggestion. However, it is of interest that all the pyrimidines found to stimulate the protein as well as deoxyribonucleic acid synthesis in plants (i.e. 6-MU, 5-azauracil, 6-azauracil) are intensive hydrogen bond donors through their 2- and 4-hydroxyl substituents and practically all of them exhibit weakly basic character, with a basic dissociation constant pK in the range of 0.5—3.0. In addition, both hydrogen bond donor and hydrogen bond acceptor ability appears to be a general character of pyrimidine herbicides.

It would be of interest to find direct chemical evidence of how pyrimidines are able to influence the secondary (or tertiary) structure of protein and of nucleic acids in living cells and in cell-free electron-transferring particles under in vitro experimental conditions.

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Prepared at the Department of Organic Chemistry of the Isotope Institute of the Hungarian Academy of Sciences, Budapest.

B. I. POZSÁR

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STUDIES ON THE BEGINNINGS OF PROTEIN DIGESTION IN NEWBORN PIGLETS

The mechanism of protein digestion develops gradually in the newborn piglet (HARDY 1969). Earlier studies (BAINTNER 1973a) have shown that the activities of proteolytic enzymes appear in a well defined sequence in the small intestine of piglets sucking colostrum and that proteolytic activities are not usually demonstrable until the piglets are half a day old.

Sow's colostrum contains a considerable amount of trypsin inhibitor, but its concentration declines rapidly in the course of subsequent sucklings, both in the colostrum (LASKOWSKI *et al.* 1957) and in the piglet's intestine (BAINTNER 1973a). Apart from trypsin, chymotrypsin A is also inhibited by the colostral inhibitor (WU—LASKOWSKI 1960) which, however, has little inhibitory influence, if any, on other intestinal proteases (BAINTNER 1974).

In view of the above findings, the question arose whether the absence of proteolytic activity during the first approx. 12 hours of life is due to the absence of pancreatic secretion, or to the presence of colostral trypsin inhibitor in the digestive system, or to both. Two experiments (A and B) were performed to obtain more information on the problem. In experiment A, proteolytic activity was determined in the intestinal contents of newborn piglets fed on cow's milk free from trypsin inhibitor. Experiment B included a larger, more heterogeneous group of piglets than previously (BAINTNER 1973a), to obtain evidence on whether the appearance of trypsin activity, i.e. the disappearance of colostral trypsin inhibitor, coincides with the vanishing of eosinophilic droplets from the enterocytes. For this purpose the examinations were carried out in the period of cessation of intestinal protein absorption (closure), i.e. from 1 to 3 days of age.

Experiment A was conducted on 39 piglets from 5 sows (litters I—V). The piglets were isolated from the sow immediately after birth. Piglets of the experimental group (24 animals) were given 20 ml cows' milk at body temperature through a gastric tube 2—4 hours after birth, and the same amount was given again 1 hour later. After another hour the piglets were killed, the small intestine was secured and, after removal of the mesentery, it was divided into a proximal and a distal half. Each part was rinsed with 5 ml saline to collect the contents in test tubes, which were frozen until use. Three piglets in each litter were provided with markings to serve as controls. Two of the three control piglets were maintained on the sow for one hour to suckle colostrum, and after another hour they were killed. The third control piglet was starved until sacrifice in all litters except litter I, in which all three control piglets were allowed to suckle the sow. Samples of intestinal contents were secured from the controls in the same manner as above. Flushing of the intestines with saline was necessary because the intestinal contents were so minimal both in artificially fed and starved piglets, that simple squeezing would not have yielded a sufficiently large sample.

In experiment B, a total of 34 piglets, originating from 4 different herds, were sacrificed between 1 and 3 days of age. The age of the animals was assessed in hours, with an exactness of ± 2 hours. Each piglet belonged to a different litter and was maintained on the sow until

Table 1

Trypsin inhibitor and proteolytic activities in the contents of the small intestine of 4—6 hour old piglets under different dietary conditions

Litter	Treatment	Serial no. of piglets	Trypsin, μg/ml	Chymotrypsin, μg/ml	Trypsin inhibitor, μg/ml
I.		1. (P + D)	4	0	0
		2. (P + D)	0	0	0
		3. P	3	35	0
	A.	D	0	35	0
		4. P	0	0	0
		D	0	0	0
		5. P	3	0	0
		D	4	0	0
		6. P	0	0	710
		D	0	0	100
	B.	7. P	0	0	920
		D	0	0	20
		8. P	0	0	210
		D	0	0	0
II.		9. P	0	22	0
		D	0	15	0
		10. P	0	24	0
		D	0	0	0
	A.	11. P	0	18	0
		D	0	38	0
		12. P	0	31	0
		D	0	16	0
		13. P	0	23	0
		D	0	6	0
		14. P	0	8	530
	B.	D	0	10	390
		15. P	0	18	390
		D	0	8	310
	C.	16. P	0	14	0
		D	0	29	0
III.		17. P	0	0	60
		D	0	0	60
		18. P	0	0	60
		D	0	0	60
	A.	19. P	0	0	0
		D	0	0	0
		20. P	0	0	0
		D	0	0	0
		21. D	0	0	0
		22. P	0	0	1450
	B.	D	0	0	1550
		23. P	0	0	1840
		D	0	0	1780
	C.	24. P	0	0	0
		D	0	0	0

Litter	Treatment	Serial no. of piglets	Trypsin, μg/ml	Chymotrypsin, μg/ml	Trypsin inhibitor, μg/ml
IV.	A.	25. P	0	0	0
		D	0	0	40
		26. P	0	0	0
		D	0	0	0
		27. P	0	0	0
		D	0	0	0
		28. P	0	0	0
		D	0	0	0
		29. D	0	0	0
	B.	30. P	0	0	840
		D	0	0	260
		31. P	0	0	660
		D	0	0	230
	C.	32. P	0	0	0
		D	0	0	0
V.	A.	The 4 experimental piglets have been excluded from evaluation			
	B.	37. P	0	0	3500
		D	0	0	1140
		38. P	0	0	1960
		D	0	0	440
	C.	39. P	0	0	0
		D	0	0	4

Contents of the proximal (P) and distal (D) parts of small intestine, each secured by rinsing with 5 ml saline, were used for the measurements. P + D indicates the entire small intestine. BAEE-splitting activity insensitive to soybean trypsin inhibitor was not demonstrable in any case studied.

A: 2×20 ml cow's milk, administered through gastric tube;
B: Suckling of sow's colostrum for 1 hour;
C: Starvation.

sacrifice. The intestinal contents of the piglets were secured by squeezing into test tubes and were made up to tenfold volume with distilled water before use. Specimens of jejunum (45%) and ileum (80—85%) were secured for histological examination. (The percentages indicate the site of excision of the specimen, taking the pylorus as 0% and the ileocoecal valve as 100%.) The specimens were fixed in formalin, embedded in paraffin, and stained with haematoxylin and eosin.

In both experiments the insoluble parts were removed from the chyme samples by centrifugation. The proteolytic activity was measured by the method of SCHWERT—TAKENAKA (1955), using benzoyl-arginine ethyl ester (BAEE) and acetyl-tyrosine ethyl ester (ATEE) as substrates. The BAEE-splitting activity was separated into an inhibitable (trypsin) and a non-inhibitable component by addition of excess soybean trypsin inhibitor (STI).

Trypsin inhibitor capacity was determined by measuring the change in activity of a known amount of trypsin in the presence or absence of the sample. The results were expressed as μg trypsin inhibited by 1 ml chyme (i.e. intestinal rinsing fluid).

For calculation of the linear correlation coefficients, the crosses (Table 2) marking the frequency of eosinophilic droplets were replaced by integers from 0 to 3. The significance of

Table 2

Trypsin inhibitor and proteolytic activities in the contents of small intestine in conventional piglets aged 1—3 days

Herd	Serial No.	Litter size	Age (±2 hours)	Trypsin, μg/ml	STI-insensitive BAEE-splitting act., μg/ml	Chymotrypsin, μg/ml	Trypsin inhibitor, μg/ml	Eosinophilic droplets	
								jejunum	ileum
	1	13	48	+	10	11	170	+++	++
	2	9	47	+	30	0	1890	+++	+++
	3	7	48	+	9	35	330	++	++
	4	7	32	+	21	9	210	—	+
L	5	5	41	0	8	14	1630	++	++
	6	9	42	0	0	10	70	+	+
	7	8	36	+	7	43	60	+	+
	8	7	52	+	8	3	110	++	—
	9	10	37	+	17	0	510	+++	+++
	10	6	53	+	17	0	250	++	++
	11	10	46	+	0	14	480	+	+
	12	10	38	+	17	0	170	++	++
	13	10	39	+	0	0	1160	++	++
	14	10	38	+	0	63	90	++	++
	15	10	38	+	20	14	40	++	++
	16	10	48	52	7	70	0	++	++
	17	10	49	+	2	0	270	++	++
K	18	10	44	0	0	0	1570	—	+++
	19	10	68	17	0	54	0	—	—
	20	10	63	38	0	55	0	—	—
	21	10	60	110	0	89	0	—	—
	22	10	64	0	0	0	90	—	+
	23	9	70	104	0	136	0	—	—
	24	9	62	0	0	0	65	—	+
	25	9	71	30	0	21	0	—	+
	26	10	72	83	0	0	0		
	27	10	63	0	0	0	60	—	
	28	10	31	0	0	29	60	+	+
Hk	29	8 → 5*	31	0	0	28	2510	++	++
	30	11	33	0	0	36	70	+	++
	31	10	60	0	0	0	30	—	+
Hh	32	12 → 11*	61	0	0	5	700	++	+++
	33	8 → 4*	60	+	0	3	500	+	+
	34	10	60	0	0	0	220	+	+

Each piglet originated from a different litter. The live weight of piglets originating from farm "Hk" was less than 1 kg at birth. (*) signifies reduction of litter size by mortality. (+) signifies trypsin activity demonstrable in the presence of excess colostral trypsin inhibitor. STI = soybean trypsin inhibitor.

The symbols indicating the frequency of histologically demonstrable eosinophilic droplets are interpreted as

— none in sample studied;
\+ sporadic occurrence;
++ moderate frequency;
+++ close to maximum frequency.

differences between values measured in the proximal and distal segments of the intestine (Experiment A) was calculated by the method of paired samples and by the t-test (SVÁB 1973).

Experiment A. Among the piglets belonging to litter V, only the controls were included in the evaluation, because the possibility that the experimental mates may have gained access to colostrum could not be excluded with full certainty.

Chymotryptic activity was demonstrable in either one or both intestinal portions of a piglets belonging to litter II (Table 1). The values ranged from 0 to 38 μg/ml. Proteolytic activity other than chymotrypsin was, however, not found in any piglet of this litter.

In the remaining litters only piglet No. 3 showed a notable proteolytic activity (chymotrypsin), while in the test of the animals trypsin was demonstrable only in traces, if at all. Chyme samples from most piglets showed no proteolytic activity at all.

In the chyme of the control piglets, which had had access to colostrum, the trypsin inhibitor concentrations ranged from 0 to 3500 μg/ml, and the values measured in the proximal portions of the intestines were significantly ($p < 0.05$) higher than those in the distal portions. The chyme of piglets given cow's milk and of those starved was free from trypsin inhibitor in all except four animals, in whose chyme it was detected at low concentrations (up to 60 μg/ml).

Experiment B. Most of the conventionally kept piglets killed between 1 and 3 days of age in this experiment had both trypsin inhibitor and proteolytic enzymes in the chyme (Table 2).

Five of the 34 piglets included in experiment B showed very high (> 1000 μg/ml) concentrations of trypsin inhibitor. These animals were all less than 2 days old. Among the 7 piglets not possessing trypsin inhibitor, 6 were older than 2.5 days and 1 was 2 days old.

Among the 34 piglets 22 had trypsin in the chyme (Table 2), usually with trypsin inhibitor also present. Trypsin was also present in the chyme of the 7 piglets noted for absence of trypsin inhibitor. The maximum values measured for the different proteolytic activities were the following: trypsin 110 μg/ml, chymotrypsin 136 μg/ml, STI-insensitive BAEE-splitting activity 30 μg/ml. No kind of proteolytic activity was demonstrable in 5 piglets of the 34.

The frequency of eosinophilic droplets was as a rule similar in jejunal and ileal enterocytes (Table 2). The frequency was less than moderate in both intestinal portions examined in 17 of the 34 piglets; most of these animals were older than 2.5 days. One 2.5 day old piglet (Hh 32) still had many eosinophilic droplets in the enterocytes, but occurrence was not more than sporadic after this age.

The following correlations were obtained between trypsin inhibitor concentrations and frequencies of eosinophilic droplets in the intestinal segments studied: *jejunum* $+0.366$ (slight correlation), *ileum* $+0.536$ (moderate correlation).

It was shown previously (BAINTNER 1973a) that the high trypsin inhibitor content of sow's colostrum is demonstrable in the intestinal content of piglets after the first suckling. Thus in the present studies the trypsin inhibitor found in the chyme of piglets that had been allowed to suckle the sow was clearly of colostral origin. Trypsin inhibitor originating from the meconium (CARLSSON—KARLSSON 1972, BAINTNER 1975) was demonstrable only in a few cases in the intestinal contents, and even then its concentration was low (Table 1).

Of the proteases, trypsin, STI-insensitive BAEE-splitting activity (in all probability identical with kallikrein) and chymotrypsin activity were determined. Since this chymotrypsin activity was insensitive to colostral trypsin inhibitor, it was clearly not identical with chymotrypsin A.

Experiment A: A comparison between conventional piglets and piglets given cow's milk. Piglets having suckled maternal colostrum showed high intestinal trypsin inhibitor concentrations after suckling for 1 hour; the level of the inhibitor was especially high in the proximal segments of the small intestine (Table 1). This finding, along with evidence of the absence of proteolytic enzymes in the early postnatal stage, accords with earlier observations (BAINTNER 1973a). (As a matter of fact, the presence of enzymes not demonstrable with the

applied methods cannot be excluded.) The piglets of litter II were an exception in respect of proteases, because all had notable intestinal chymotryptic activities.

The piglets deprived of cow's colostrum and fed on cow's milk had only very low concentrations of trypsin inhibitor in the chyme, if any, and the proteases were also missing. The piglets of litter II, and some of litter I were exceptions in this respect. However, only one type of proteolytic activity, which was also shown by the control animals, was found in litter II. In view of the foregoing considerations it is concluded from experiment A that the absence of proteolytic activity in piglets during the first postnatal hours was for the most part due to deficiency of pancreatic enzyme secretion, the role of colostral trypsin inhibitor being chiefly auxiliary in this period, i.e. the protection of colostral antibodies during absorption.

Experiment B: Studies on piglets from 1 to 3 days of age. The nutrition, digestion and absorption of piglets changes considerably between 1 and 3 days of age. During this period sow's colostrum transforms to transitory milk, the absorption of undigested proteins ceases (closure), the eosinophilic droplets disappear from the enterocytes, and the colostral trypsin inhibitor vanishes from the intestinal content, while trypsin activity begins to make an appearance there. The time course of the latter three events was pursued in the present study, partly to detect possible further interrelationships.

The histological study of eosinophilic droplets was used for a follow-up of protein absorption. Protein absorption declines rapidly in piglets during the second day of life until it practically ceases at the age of 1.5 to 2 days (NELSON 1932, HOERLEIN 1952, BARRICK *et al.* 1954, BRAMBELL 1958, SPEER *et al.* 1959, OLSSON 1959, LECCE—MATRONE 1960, LECCE—MORGAN 1962, LECCE *et al.* 1964, MÖHRING—STRUNZ 1968). Traces of it are, however, still detectable in adults (WALKER 1975). The conflicting views expressed in the literature on this point may well stem from the dissimilarity of the arbitrary thresholds below which protein absorption is regarded as negligible. If protein absorption is followed up by the histological demonstration of eosinophilic droplets inside enterocytes rather than by immunological procedures or radioactive labelling, the results may well be dissimilar, because each approach detects a different phase of the process.

In the present experiment the frequency of eosinophilic droplets clearly declined with advancing age despite considerable variations. Neither maximum nor moderate frequency could be observed after 2.5 days of age. The closure time so assessed is associated with a somewhat later age than the 1.5—2 days reported by most authors.

In the present study, the occurrence of eosinophilic droplets was quite rare in specimens taken from animals older than 2.5 days; the few droplets found might not notably contribute to protein transport, since in all probability they signified delayed discharge of proteins into the circulation by some enterocytes. By 3 days of age even these droplets disappeared, or were transformed into tiny eosinophilic inclusion bodies in the ileum. The inclusions resided in liquid-filled vacuoles and were demonstrable for several subsequent weeks within the enterocytes of the ileum (BAINTNER 1973b).

The concentration of colostral trypsin inhibitor (CTI) fluctuated considerably in the chyme. The CTI vanished at approximately 2 3/4 days of age, which corresponded roughly with the time of disappearance of the eosinophilic droplets. Nevertheless, a comparison of the two parameters by linear correlation resulted only in slight *(jejunum)* or moderate *(ileum)* correlations, indicating a more intricate interrelationship than a simple cause-effect relation, as already concluded from pertinent earlier investigations (BAINTNER 1973b). This conclusion does not, however, exclude the assumption that CTI may promote the development of eosinophilic droplets in the early postnatal period when the intestinal epithelium is still able to absorb proteins.

Trypsin activity was demonstrable in the chyme well before the disappearance of CTI, not infrequently in face of a considerable excess of inhibitor. A possible explanation is that

in such cases a considerable amount of trypsin-CTI complex was present, part of which became dissociated to trypsin and CTI under the conditions of high dilution required for the trypsin assay. For this reason the trypsin values determined in face of CTI-excess have not been regarded as realistic, and their numerical values have not been included in this paper. On the other hand, CTI-determinations were clearly realistic even under these conditions, because CTI is always measured in the presence of trypsin added in excess, which depresses the dissociation of the trypsin-CTI complex. Thus the results represent the quantity of CTI that is in excess of the trypsin produced by the animal. The quantitative determination of the trypsin-CTI complex was not possible for lack of an appropriate method.

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*

Prepared at the Department of Physiology, Institute for Animal Breeding, Budapest.

K. Baintner jr.

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FORUM

LIQUID INVERT SUGAR MANUFACTURING

PARTICIPANTS

ALMÁSI, E. *professor* (University of Horticulture, Department of Food Technology and Microbiology, 1118 Budapest, Ménesi út 45.)
BAJNÓGEL, J. *director* (Research Institute of Home Trade, 1051 Budapest, Vigadó u. 6.)
BARDACH, S. *chief official* (Ministry of Food and Agriculture, Department of Food Industry 1055 Budapest, Kossuth Lajos tér 11.)
BENDE, P. *chief engineer* (Szabadegyháza Distillery, 2432 Szabadegyháza)
BODNÁR, M. *assistant professor, head of department* (Debrecen University of Agricultural Sciences, Department of Agricultural Economics, 4001 Debrecen, Böszörményi út 138.)
BOLGÁR, P. *technical manager* (Distilleries Trust, 1089 Budapest, Diószeghy S. u. 8.)
FAZEKAS, S. *assistant lecturer* (Semmelweis Medical University, 2nd Institute of Chemistry and Biochemistry, 1088 Budapest, Puskin u. 9.)
FÜREDI, J. *assistant professor* (Gödöllő University of Agricultural Sciences, Department of Plant Breeding, 2103 Gödöllő)
GALLÓ, GY. *chief engineer* (Pécs Starch Factory of the Szabadegyháza Distillery, 7601 Pécs, Bolgár Néphadsereg u. 47.)
GÁBOR, M. *professor* (College of Food Industry, Department of Chemistry, 6701 Szeged Marx tér 7.)
GÁSPÁR, L. *scientific head of department* (Agricultural Research Institute of the Hungarian Academy of Sciences, 2462 Martonvásár)
GYŐRVÁRI, I. *assistant professor, head of department* (Gödöllő University of Agricultural Sciences, Department of Agricultural Economics, 2103 Gödöllő)
HOLLÓ, J. *academician, director* (Central Chemical Research Institute of the Hungarian Academy of Sciences, 1025 Budapest, Pusztaszeri út 59—67.)
HORVÁTH, GY. *technical consultant* (Szabadegyháza Distillery, 2432 Szabadegyháza)
KATONA, J. *chief director* (Research Institute of Viti- and Viniculture, 1022 Budapest, Herman O. u. 15.)
KÁDÁR, GY. *professor* (University of Horticulture, Department of Oenology, 1118 Budapest, Ménesi út 45.)
KISFALVI, T. *research worker* (Research Institute of Agricultural Economics, 1355 Budapest, Zsil u. 3—5.)
LÁSZTITY, R. *professor* (Budapest Technical University, Department of Biochemistry and Food Technology, 1111 Budapest, Műegyetem rkp. 3.)
LŐRINCZ, J. *professor* (Gödöllő University of Agricultural Sciences, Department of Soil Cultivation and Plant Production, 2103 Gödöllő)
MAJER, J. *assistant lecturer* (Gödöllő University of Agricultural Sciences, Department of Agricultural Chemistry, 2103 Gödöllő)
NAGYPATAKI, I. *director* (Szabadegyháza Distillery, 2432 Szabadegyháza)
NÉMETH, S. *deputy director* (Research Institute of Irrigation, 5540 Szarvas, Szabadság u. 2.)
ÖRÖSI PÁL, Z. *scientific head of department* (Research Institute for Small Animal Breeding, Apiculture Department, 2101 Gödöllő)
PAIS, I. *professor* (University of Horticulture, Department of Chemistry, 1114 Budapest, Villányi út 29—31.)
PÁL, GY. *editor* (Editorial Office of Acta Agron. Hung., 2462 Martonvásár)
PÁSZTOR, K. *assistant professor* (Debrecen University of Agricultural Sciences, Department of Plant Production, 4001 Debrecen, Böszörményi út 138.)

PECZNIK, J. *professor* (Gödöllő University of Agricultural Sciences, Department of Agricultural Chemistry, 2103 Gödöllő)
PÉNZES, L. *senior research worker* (Semmelweis Medical University, Research Group on Gerontology, 1085 Budapest, Somogyi Béla u. 33.)
POZSÁR, B. *senior research worker* (Isotope Institute of the Hungarian Academy of Sciences, 1525 Budapest, Konkoly Thege út)
RIGÓ, J. *director* (National Institute of Dietetics, 1085 Budapest, Makarenkó u. 24.)
ROMÁNY, P. *minister* (Ministry of Food and Agriculture, 1055 Budapest, Kossuth Lajos tér 11.)
RUFF, J. *head of production section* (Ászár Starch Factory, 2881 Ászár)
SHMILLIÁR, M. *retired director* (Research Institute of Sugar Production, Beet Growing Research Station, 9463 Sopronhorpács)
SÓLYOM, L. *director* (Research Institute of the Distilling Industry, 1089 Budapest, Diószeghy S. u. 8.)
SZÁNTÓ, S. *laboratory director* (Research Laboratory of the Hungarian Confectionery Industry, 1097 Budapest, Vágóhíd u. 20.)
SZENDREY, I. *professor* (University of Forestry and Wood Industry, Department of Chemistry, 9401 Sopron, Bajcsy-Zsilinszky u. 4.)
SZÉKÉSSY-HERMANN, V. *professor* (Semmelweis Medical University, 2nd Institute of Chemistry and Biochemistry, 1088 Budapest, Puskin u. 9.)
TARJÁN, R. *director* (National Institute of Nutrition, 1097 Budapest, Gyáli út 3/a.)
TYIHÁK, E. *research worker* (Research Institute for Medicinal Plants, 2011 Budakalász, József A. u. 68.)
VUKOV, K. *professor* (University of Horticulture, Department of Canning Technology, 1118 Budapest, Ménesi út 45.)
ZELLER, GY. *assistant professor, head of department* (Karl Marx University of Economics, Department Group for Market Organization, 7601 Pécs, Rákóczi u. 80.)

*

Prepared in 1977—1978.

PÁL, GY.: **The continental blockade of the Napoleonic times was in great part responsible for the setting up of sugar manufacturing in Europe, and thereby for the appearance of sugar-beet, a new cultivated plant supplying saccharose. Nowadays does the manufacture of invert sugar from maize mean the discovery, or more correctly the rediscovery, of a plant supplying a new sugar raw material, though not saccharose, or is it only a temporary solution for preventing the shortage and price increase of sugar on the world market?**

ALMÁSI, E.: Invert sugar production from maize is, in my opinion, not a temporary solution to prevent the shortage and price increase of sugar.

In the United States the production of liquid sugar from maize has become established and is steadily progressing in spite of the possibility of producing sugar either from sugar-beet or from cane.

The complex industrial processing of maize also renders it possible to manufacture other products, which are important for the national economy but have in part had to be imported so far.

BAJNÓGEL, J.: The use of maize for invert sugar production is neither a new discovery nor a rediscovery; it is rather a question of applying earlier knowledge. The production of invert sugar from starch is a practice which has been known for some time. Maize is a raw material for starch and can be reckoned with as an excellent possibility.

Invert sugar manufactured from maize has become a subject of great interest because of the sugar shortage and the consequent increase in sugar prices. In other words, it was economic necessity rather than a consumer demand for invert sugar that raised the idea of producing sugar from maize. I think that under our present conditions the consumption of invert sugar should be considered as only a temporary solution for the households, or simply as an attempt to find a new way out.

BARDACH, S.: For my part, instead of putting the question in the form of "new raw material for sugar production, or a temporary solution to make up for the sugar shortage" I would suggest a different approach to the necessity of development.

The welcome progress recently made in the field of maize production, the favourable ecological conditions in Hungary and the traditions of maize growing have made

the maize plant one of the most important crops in Hungarian agriculture. The Hungarian national economic plan aims at a yield of about 7.5—8.0 million tons in the near future and according to long-term predictions the yield will continue to increase. The total value of the above volume is some 20—25 thousand million Ft, which is of an order of magnitude equal to the annual production of the mining or the electrical energy industries. On this basis I think it is quite right to regard maize as one of the most important and practically inexhaustible raw material sources of the Hungarian national economy.

The maize produced (by which maize grain is to be understood, except during the discussion of the by no means small possibilities inherent in the utilization of the whole maize plant or of cobs and stalks) is mostly used today, and probably in the future, for fodder. With the volume produced in excess of this, however, Hungary has recently appeared as an exporter on the world market.

According to the predictions the volume of maize exportable by Hungary is expected to increase by about half a million tons during every five year period, and reach 3—4 million tons before the turn of the century.

The world market price for maize grain is expected by Hungarian foreign trade experts to become stabilized for some time at about 110.0 $/ton.

This price level shows the export of unprocessed maize grain to be favourable, the more so because the industrial investment demands (storage areas, transportation) are relatively low.

This standpoint, which in itself is correct and up-to-date, is, however, opposed by the following facts:

— It is a basic economic rule that marketing the products of high level industrial processing is always more profitable than selling raw material;

— When unprocessed maize is exported a considerable amount of protein, imported in fact from capitalist countries, and vegetable oil, which is easy to export, also leaves the country. The above considerations explain the desire to utilize a certain proportion of Hungary's maize surplus for industrial purposes.

As well as the economic aspects, which are always a primary consideration, the possibility of rapid achievement, the aim at complexity in the processing and the demand structure also determine the objectives of industrial processing.

During recent years Hungary has been compelled to purchase considerable volumes of sugar, alcohol and protein feed on the capitalist markets. It is for this reason, and not exclusively because of the sugar, that a method of processing by which all three of these products can be obtained from maize has been chosen.

BENDE, P.: Sugar production from maize, like that from sugar-beet, is not a new practice. The composition of the sugars obtained from maize, however, has only recently been successfully made similar in its chemical and physical properties to that of beet sugar solution. This has considerably increased the possibilities for the industrial utilization of liquid sugar produced from maize.

BODNÁR, M.: The possibility of producing invert sugar from maize is definitely a noteworthy circumstance as regards the future sugar supply for the world population. In my opinion it is not merely a temporary solution, but means the extension of the raw material basis of sugar production.

BOLGÁR, P.: The manufacture of liquid sugar from maize can be regarded as a new source of sugar for consumption.

FÜREDI, J.: The manufacture of invert sugar from maize is not, in my opinion, a temporary solution, nor is it a mere attempt to make up for the shortage of sugar on the world market. Compared to granulated cane and beet sugar. liquid invert sugar has various advantages from the point of view of the preserving industry and the confectionery industry.

GALLÓ, GY.: With the industrial production of maize the yield average per unit area has substantially increased. Its use for feeding purposes alone is not economical. Its utilization as an industrial raw material has gained ground in recent years. Its use for liquid invert sugar production cannot be regarded as a temporary solution; it will be maintained until a more economic way of producing sugar is discovered.

GÁSPÁR, L.: In my opinion the manufacture of invert sugar from maize cannot be compared to the nearly two hundred year career of sugar-beet and beet sugar, which have fundamentally determined the development of agriculture in Europe. It is not, in fact, a new discovery in agriculture, nor is it a question of introducing a new crop. In big maize growing countries like the United States of America maize was first processed on an industrial scale into glucose, maltose and malto-dextrine (corn syrup, corn sugar, cerelose, anhydrous dextrose, dextrose hydrate) in the forties, when the confectionery and soft drink industries used about 3,000 tons of corn sugar. This quantity was, however, only 2.5% of the total amount of cane and beet sugar used. The development of glucose production following the work of the German chemist Kirchoff was in fact initiated by the same historical event, the continental blockade of the Napoleonic times, as that of sugar beet. However, while in Europe, where maize production was at that time insignificant, potato starch became the raw material of glucose production, in America maize starch provided the basic material.

The volume of glucose production is determined by the demand. In the confectionery and soft drink industries glucose can be substituted for saccharose only to a limited extent. It is known that taking the "sweetness" of saccharose as 100, the relative sweetness of D-glucose is 74, and that of D-fructose 173. Certain properties of glucose, which have an unfavourable infleunce on the physical consistency of confectionery products, do not recommend it for industrial utilization either. It is thus easy to understand that interest was at once aroused when, during an economic period unfavourable for cane and beet sugar but favourable for maize, science and industry came forward with invert sugar produced from maize. The interest was all the greater because this product was essentially identical to the sugar used earlier in a number of industries. In the course of manufacturing jams, stewed fruit, soft drinks, and in other confectionery technologies as well, saccharose becomes more or less inverted. So there is no essential difference between the old product and the new one.

However, this important expansion of the utilization of maize by the sugar industry has undoubtedly been made possible by an achievement ranking as a discovery, which forms part of a development trend in the chemical industry characteristic of our age. The isomerization of glucose into fructose with a high degree of economic efficiency is a very important result in the biochemical industry. It is worth mentioning that the chemical pathway itself has been known for a long time, as Lobry de Bruyn and van Ekenstein, in 1896 and 1897, studied the phenomenon whereby D-fructose is produced from D-glucose treated with low concentration acids in media free from air and oxygen due to the isomerization aldose $\rightleftharpoons$ ketose, which involves a rearrangement in carbon atoms 1 and 2. However, this well-known process of carbohydrate chemistry was never made use of industrially, obviously because of technical difficulties.

GYŐRVÁRI, I.: Invert sugar production cannot be the consequence of a price increase on the world market, neither can it be an attempt to check the price increase. Basically we cannot speak of the temporary shortage of sugar as a price-increasing factor, at least not as one causing a greater price increase than what has been experienced from time to time with wheat and other fundamental foodstuffs. During the past fifteen years there was an abnormally large, though very short-term, price increase on two occasions, in 1963—64 and 1974—75. In both cases, although the current predictions indicated that a relative yield surplus could be expected, a speculative price increase was started by a fall-off in the supply amounting to a few hundred thousand tons. This resulted in the first case in a nearly five-fold, and in the second case in an almost seven-fold price increase within the crop year (from September to August). The boom sugar prices of the early sixties were followed by a sharp price drop which brought down the world market price of sugar almost to the level it had been at the beginning of the century, where it remained until the early seventies. The upswing in the middle of the seventies was very short-term, and even the 1976 prices were not higher than would have been justified by the general increase in prices. Such short-term and infrequent price increases cannot be sufficient reason for setting up a new process for sugar production: the 1974 price peak did not last long enough for an industrial-scale production technique to be developed. The process started earlier, and the conditions for industrial production happened to mature just at that time in the United States.

HOLLÓ, J.: High-fructose liquid sugar obtained from maize can be considered as a new basic raw material for sugar, involving not only a temporary solution to the sugar problem but the introduction of a new branch of industry. Beet sugar was not a "new type of

cane sugar" as it is, in fact, chemically identical with the latter. It has long been known that starch can be decomposed into its basic components. Recently, however, with the appearance of a new enzyme, isomerase, about half the glucose formed can be inverted into fructose, which was previously not an economical process.

HORVÁTH, GY.: Some 60 years after the chemist Margraaf discovered the presence of sugar in beet, the manufacture of sugar from beet started in Europe. Since then the sugar content, yield and sowing area of sugar-beet have substantially increased. However, the total amount of cane and beet sugar is unable to satisfy the demand for sugar on the world market.

The price increase of sugar until recently can be attributed to this. It follows that manufacturing invert sugar from maize as basic material is not a temporary solution to make up for the shortage of sugar, but a final solution which will prevent sugar prices from increasing.

But the fact that maize is able to fulfil this important task is not a recent discovery or rediscovery. The constant development of the quantity and quality of products made from maize starch has made maize indispensable in the modern nutrition of the world. In this development the most important milestones were the following:

In the middle of the last century the firm of Kingford and Durey in the United States of America developed a wet starch processing method (corn wet milling process) which produced very good quality starch. The United States has possessed the world's largest starch industry ever since.

Until 1938 corn syrup was produced from starch obtained by wet processing with acid hydrolysis; it is widely used even today in food products such as bread, the taste, smell, texture and the colour of the crust of which are favourably influenced by it. It is used in canned fruit, jam, icecream, sweets, jellies, canned meat, soft drinks, etc. This corn syrup had a DE (Dextrose Equivalent) value of 30—56; its main deficiency from the point of view of sweet fruit conserves was the absence of a sweet taste, and from the point of view of fermented foodstuffs (e.g. bread) the low value of fermentable carbohydrates.

The acid-enzyme conversion process was introduced in 1938; the approx. 52 DE product of the acid hydrolysis was neutralized, and further converted by means of an enzyme system. The sweetness and fermentability of the 62—64 DE product thus obtained was already much more favourable, and syrup itself was easier to handle (more favourable viscosity and a lower tendency to crystallize).

It was at the beginning of the fifties that the glucoamylase enzyme was first produced in commercial quantities; with this corn syrups can be converted to 95—97 DE. This high DE glucose solution is readily crystallized at low temperatures, while at higher temperatures it shows good storability, and is sweeter and easier to ferment than the 62—64 DE corn syrup.

Years later the starch industry aimed at developing corn syrups even sweeter than those produced so far. The obvious solution seemed to be to produce syrups containing a considerable amount of fructose, since fructose had long been known to be the sweetest sugar among the mono- and disaccharides.

Accordingly, in 1964 a trial production of the enzyme capable of converting glucose into fructose was begun in the United States. The operation was performed at the American Clinton Corn Processing Company (Subsidiary of Standard Brands Incorporation). In 1965 they became acquainted with a Japanese discovery, by which a considerable amount of glucose-isomerase enzyme could be produced with the use of Streptomyces bacterium cultures. Realizing the importance of this discovery, the Clinton Company conducted successful negotiations with the Japanese Ministry of International Trade and Industry to acquire the exclusive rights to a Japanese licence in this matter.

The contract was drawn up, and since then much progress has been made:

At the beginning, in 1967, a syrup with a 14% fructose content was produced under the trade name Isomerose^R 30 Brand. But by 1968 Isomerose^R 100 syrup was developed, in which the fructose component reached 42% of the dry matter content, leaving only 50% glucose. On a dry matter basis this product is equal in sweetness to beet sugar, and was granted the Putnam Award in 1971.

Later, in 1973, the isomerase enzyme was immobilized on an insoluble carrier. This causes the isomerization to take place continuously within minutes, or at the most in a few hours. In 1975 the continuous isomerization process for glucose syrup was again granted the Putnam Award.

Sugar syrup with this composition was first produced in Europe on a commercial scale by the Dutch company Koninklijke Scholten Honig N. V. Amsterdam, using Reynolds' isomerase enzyme.

A maize sugar factory suitable for processing 300,000 tons maize/year is under construction near London, the product of which will also have a 42% fructose content.

In the German Federal Republic, in factories belonging to the Maizena Group, large volumes of maize sugar syrup containing 42% fructose are produced.

Naturally, the Japanese have since developed a new, better isomerase enzyme bound to an absolutely insoluble carrier. Their high fructose corn syrup production is of international fame.

This liquid invert sugar competes with beet and cane sugar on the world market, especially in Belgium, Holland, England, the German Federal Republic and the United States of America.

The main field of competition is the wholesale price. The favourable wholesale price of maize sugar has created a very adverse situation on the western sugar market for beet and cane sugar by consolidating their prices.

At the same time it means considerable savings in sugar imports for the United States, since it is able to replace beet and cane sugar in all industries in which sugar is used.

According to the most recent information the Clinton Corn Processing Company is today able to produce 90% granulated fructose.

This is a brief historical review of invert sugar manufacturing from maize, which answers the problem raised in the question.

KISFALVI, T.: The current and expected further development of the production of large volumes of invert sugar from maize depends on the simultaneous action of a number of factors.

The storage of sugar on the world market and the increase in its price is only one of these factors.

Further factors are the very large volumes of maize grown in one country, the discovery of the enzymes required for up-to-date invert sugar manufacturing and how to use them in mass production, the considerable saving of specific energy involved with up-to-date invert sugar production, the lower specific raw material transport costs in the case of maize grain, the specific cost-reducing effect of the by-products, or more correctly twin-products: high value concentrated protein feed and germ oil for human consumption, the reduction in specific amortization costs due to the fact that the production capacity can be utilized all the year round, and the elimination of water pollution, which occurs during beet sugar production.

The maize yield average in the United States of America was 17 q/ha in 1930 and rose to 60—65 q/ha by the first half of the seventies. Therefore, by this latter date nearly half of the world maize crop, amounting to more than 300 million tons, was harvested in the United States, on an area not quite a quarter of the total maize area of the world. This suddenly increased volume of maize was more than that required for livestock farming; some of it was exported as maize grain raw material, and the necessity of economical industrial processing, other than fodder production, became more and more imperative.

The processing of maize into sugar started in the United States in the mid-seventies with an annual sugar production of half a million tons; this capacity will increase to over million tons in 1977.

Ever since the starch and fermentation industries have existed it has been known that sugar can be produced from maize. The question of whether it is worth producing sugar from maize is decided in Hungary, as elsewhere in the world, by the availability of the necessary amount of maize, and the comparative price trends of meat, sugar, protein feed of vegetal origin, germ oil and maize.

Taking all this into consideration it seems probable that the manufacture of invert sugar from maize is not a temporary solution either in Hungary or elsewhere in the world.

LÁSZTITY, R.: The change in the world market prices of cane and beet sugar has undoubtedly influenced the development of invert sugar production from maize, but it would not be right to attribute the progress made so far exclusively to this cause. The manufacture of sweeteners (dextrose, starch syrup, glucose) from starch (or raw materials containing starch) can look back on quite a long past. The utilization of these products is on

the increase (manufacture of soft drinks, confectionery industry, etc.). The possibility of invert sugar production is based on the rapid progress made in the production and industrial application of enzymes, i.e. in the field of enzyme engineering.

The utilization of enzymes bound to carriers is of particular importance, since the procedure makes the industrial use of enzymes much more efficient and economical. Finally it should be noted that the technological development of the potential industrial users has also contributed to the increase in the demand for liquid invert sugar.

Taking into consideration all these factors, together with the numerous advantageous properties of maize as a raw material, the production of sugar from maize, especially in countries which grow maize and are interested in its complex utilization, cannot be regarded as a temporary or transitional solution.

LŐRINCZ, J.: The production of invert sugar from maize is also carried out in other parts of the world (e.g. in the United States). Since our experiences are so far only of an experimental character, I think it necessary to build a factory in Hungary where large-scale experiences can be acquired. The results will then decide whether invert sugar production should be introduced alongside the traditional method of producing sugar.

NAGYPATAKI, I.: It is a fact of economy history that the extension of beet-sugar production and processing transformed European agriculture and exerted a considerable effect on the development of the food industry and on nutrition, because it greatly changed the sowing structure, provided employment and income, stimulated the technical development of the food industry and provided richer and more varied nutrition for the population.

If it is true that sugar-beet production changed the agriculture of Europe in this way and exerted a strong effect on its food industry, then it is also true that maize will transform the agriculture and food production of the whole world in an incomparably greater measure. Of course, this is not only so because sugar-beet can only be grown in the temperate zone, which means that in tropical regions its role is played by sugar cane, but also because, taking into consideration the composition of maize (carbohydrate, protein, oil), the many ways in which it can be utilized, the large volume of annual yield, the fact that it yields the highest food value per unit area with the lowest specific input, and the great reserves of its genetic potential, maize must be regarded as the most important raw material resource of agricultural origin available at present in the world.

It is a long time since Christopher Columbus brought some yellow seed into Europe among the treasures given to him by the ruler of the Aztecs. The value of the seed was not realized then at the court of the Spanish king, and certainly no-one thought that this was the greatest treasure given to the Old World by the New World, yet for centuries these seeds formed the basis for the nutrition of many millions of people in Europe.

It is true that maize production spread very slowly, but in those areas where it was introduced it became the most important cultivated plant and won the name "king of cereals".

Maize is, in fact, a plant of tropical origin, which makes the best use of sunshine and moisture. This is why, of all known cereals, maize gives the largest yield per unit area, substantially exceeding even the high yielding wheat. On the basis of sporadic but easily evaluated yield data the genetic potential of maize is estimated to be at least 20 to/ha, which indicates the enormous reserves of yield potential.

Apart from this maize is a food and feedstuff of high calorific value, which can be consumed or used in more than 100 forms.

In the history of food production one of the greatest successes has been the realization of the mass production and utilization of maize. This has greatly changed food production conditions and nutrition possibilities, while giving great impetus to scientific research and to the application of new up-to-date technical and scientific achievements in the fields concerned.

In addition, it has caused a large-scale development of the supporting industries, and improved the supply of up-to-date basic and auxiliary materials to industries producing consumer goods to an extent never experienced before.

It can easily be seen that the more than 329 million tons of maize grown in the world in 1976/77 is one of the most important raw material resources of the human race, especially if we consider that its volume could be considerably increased, primarily

through higher yields but also by extending the maize growing area at the expense of crops of lower value, and also if we consider that it forms the basis of meat, egg and milk production.

In many parts of the world maize is a staple food of the population; it is also used for the production of farinaceous products, starch syrups for confectionery purpose, granulated sugar (crystalline dextrose), liquid sugar (glucose-fructose), starch, dextrines, spirits, alcohol, beer, pharmaceutical products, etc.

True, the larger part of the maize crop is still fed directly to animals, which considering the up-to-date possibilities of utilization, is very wasteful, but the first steps towards highly promising complex utilization have now been taken.

This complex utilization can be realized through the intermediary of industrial processing, where the useful components of maize: carbohydrate, protein and oil, are separated both from the ballast materials and from each other, in a concentrated from, and used either directly for human consumption or for industrial purposes.

The experience obtained so far in the utilization of maize show that although it is a feedstuff of high value because it contains large quantities of all the components carbohydrate, protein, fats) which are indispensable from a biological point of view in animal feeds, the natural proportions of these components in maize do not meet the composition requirements for modern fattening and dairy concentrates, poultry feeds, etc. at all.

It is therefore worth-while subjecting maize to industrial processing before it is used for feeding purposes, in order to obtain the individual components (carbohydrate, protein, oil), separately in a concentrated form, which can then be used partly for human nutrition and partly for human nutrition and partly as supplements mixed at proper ratios in special feeds.

Accordingly, in order to ensure the most favourable economic results, the industrial processing of maize (the production of starch, starch syrups, dextrines, maltirons, enzymes, vitamins, alcohol, dextrose, liquid sugar, eating oil, corn steep liquor, etc.) is often combined with the production of substances used for production of substances used for feeding (gluten, maize oil cake, maize bran, fodder yeast, dried feed obtained from maize stillage).

With reference to other possibilities it may be mentioned here that starch produced in the course of industrial processing is utilised in large quantities by the paper and textile industries.

Dextrose is the basic ingredient for sorbite, i.e. vitamin C production, and for enzyme production. Starch syrups and maltirons are widely used in confectionery and pharmaceutical factories. Corn steep liquor is used as medium in the production of important drugs. The enzymes are used in detergent, starch, alcohol and beer production. Alcohol is required not only for the production of beverages, but to a much greater extent in the pharmaceutical industry, vinegar production, cosmetics, household chemistry and therapeutics; it is also a valuable propellant.

It is characteristic even today to find that in many parts of the world people are short of these outstandingly important products which could be obtained from maize very economically in any quantity desired. With a view to a permanent and balanced supply of these products and to a fulfilment of the demands of the industries concerned, it seems reasonable that countries where abundant raw material is available should set up modern maize processing plants within the framework of preferential development programmes, in the same way, for example, as the petrochemical industry has been established on the basis of crude oil.

But more than this is happening: countries situated in the maize growing zones are establishing, in a non-uniform, contradictory but more and more perceptible manner, up-to-date systems of maize production and utilization, in the same way as vine cultures have been brought into existence in many countries.

Maize production and utilization should naturally be based on scientific results and carried out at the highest possible technical and technological level. However, since the quantity of maize available is much greater than that of grapes, while the production of maize is substantially cheaper than the cultivation of grapes, and since the products obtained from maize are more numerous and economically much more important that those produced from grapes, the production and utilization of maize should be organized on an incomparably larger scale.

Before very long maize will be as important from the point of view of food production and nutrition as the hydrocarbons are today in the energy supply and the chemical industry.

Maize is thus no longer just one of the many agricultural crops but is an increasingly important basic material of food production.

The new up-to-date processing methods and the possibility of multipurpose processing and utilization give a further impetus to maize production, which can be extended primarily by increasing the yield averages, but also by increasing the maize growing area at the expense of less valuable crops which have higher production and processing costs.

Owing to its position in the world economy and the possibilities latent in its cultivation, processing and utilization, maize is gradually becoming a strategic product of increasingly determinative character, used by governments within the framework of a considered policy for the deliberate adjustment of the economic structure, the intensive improvement of the domestic supply and the establishment of export markets; that is, to achieve a positive foreign trade balance and to give aid to developing countries.

It is certainly worth paying attention to the fact that while the true value of maize and the wide range of products obtained from it has only recently been recognized in the world economy, the biological, technical and economic conditions already established have resulted in very rapid progress in the production and utilization of maize. From the above it is obvious that maize is not an incidental, temporary raw material, but an agricultural crop whose high yield under favourable cultivation conditions, many-sided utilization and favourable production, storage, transportation and processing costs bring into question the competitiveness of sugar-beet, sugar cane, certain fodder grains and oil crops, and various raw materials of the starch, distilling and brewing industries, while also greatly extending the raw material basis of the products obtained from it.

NÉMETH, S.: The manufacture of invert sugar from maize is, in my opinion, not a temporary solution aimed at putting an end to the storage of sugar on the world market. The production of sugar from maize widens the range of utilization of this crop, and for economic reasons its importance will increase in the future, at least in Hungary.

PAIS, I.: I do not consider the manufacture of sugar from maize to be a temporary or emergency solution, but rather a possibility which may play an important part in the development of the food industry.

PÁSZTOR, K.: I regard the use of maize for invert sugar production as a permanent rather than a temporary solution, since it is a question of satisfying the requirements not only of public food supply but of the industries too. Knowing the prospects and limits of any further increase in the sowing area and yield of sugar-beet I think that besides the numerous advantages of maize which are so far unexploited this plant will provide a firm basis for sugar production. Furthermore, it must also be taken into consideration that it will be possible to double the present maize yields within a short time. The new prospective varieties are able to produce grain yields of more than 150 q/ha. Such a rapid increase in yield cannot be imagined in sugar-beet.

PECZNIK, J.—MAJER, J.: Sugar production from maize cannot be regarded as a temporary solution for the following reasons:

— For several years the world production of saccharose, apart from annual crop fluctuations, has been maintained at practically the same level. True, the sugar reserves have increased to some extent because the total sugar consumption was 80.4 million tons in 1975/76 compared to a world sugar production of 82.8 million tons, but the world demand for sugar is expected to amount to 107 million tons by 1985. This 2.5 million tons increase in the reserves is however expected to contribute to the stabilization of sugar prices.

— For several years Hungary has had to import a considerable amount of sugar. On the basis of the present consumption the national economic plan aims to produce the full domestic requirements by 1980. However, a further increase in the per capita sugar consumption must also be reckoned with.

— From a biological point of view the invert sugar produced from maize can be regarded as being at least as valuable as saccharose, while its utilization in the food industry may result in technological advantages.

POZSÁR, B. I.: Besides sugar cane, sugar-beet has also gained outstanding importance in the temperate zone. Sweet sorghum is expected to assume a similar role, as it can be grown not only in the temperate but also in the tropical zone. The technology based on maize grain will no doubt spread in Europe too, and it is highly probable that still further agricultural products will be used to produce basic materials for the confectionery industry, e.g. Jerusalem artichoke tubers.

ROMÁNY, P.: The invert sugar produced from maize will, in my opinion, take a firm hold of the food market; thus it is not a question of "fashion". This is likely due to a large number of positive features in the production and utilization of liquid sugar. Labour productivity in the invert sugar factory is many times higher than in the traditional sugar factory, not to mention the energetic aspects.

The world sugar market has been seen for several years to be rather unstable. Globally, taking into consideration the countries of the Third World too, the sugar deficiency is just as serious as the protein deficiency, yet the world sugar market is struggling with overproduction problems. 1976 was the year of the sugar price breakdown, and it is easy to imagine that if an overall adjustment of the raw material and food market is delayed, we shall witness the development of a "sugar cycle".

These considerations, while they undoubtedly promote the organization of large-scale invert sugar manufacturing, were not its fundamental reasons. The main reasons, I repeat, were the low energy demand, the much higher labour productivity, the complex utilization of raw materials and the variability of the end product. (With the technology available, besides sugar, the factory can also produce alcohol, the foreign exchange earning index of which is very good.) All these advantages are only partly "counterbalanced" by the fact that sugar-beet produces a higher yield per unit area than maize.

RUFF, J.: The utilization of maize as a basic material of sugar production is of quite recent origin. To start with starch produced from maize was used mainly to replace the ever decreasing amount of potato-starch and the products produced from it (starch syrup, dextrin, etc.).

Starch production from maize has now assumed considerable proportions all over the world. The invert sugar produced from maize cannot thus mean the rediscovery of maize, only a widening of the range of products obtained from it, since starch, starch syrup and dextrose, products which are widely used and sought after, can be obtained from the production line as intermediates according to the demand.

For this reason it cannot be regarded as a solution aimed at preventing the shortage and price increase of sugar on the world market, as it may occasionally take part in overproduction, although in Hungary the primary aim of the future factory is to satisfy the sugar demand of the big industrial consumers.

SHMILLIÁR, M.: Owing to the growth of the population and the increase in the per capita consumption the volume of sugar used is steadily rising. This rise is of such an extent that the traditional cane and beet sugar producing areas are no longer able to satisfy the increasing demand. The sugar cane and sugar-beet areas could still be increased, but only to a limited extent. The utilization of other suitable plants or plant products for sugar production is justified. Maize, which has a high starch content and is grown in large quantities, is one of the crops suitable for this purpose. Under Hungarian conditions maize is simpler and more reliable to grow, requires less investment and is less sensitive to ecological factors than sugar-beet.

It must be noted, however, that liquid invert sugar cannot always be substituted for beet sugar. Sugar can only be replaced by syrup with a high fructose content within certain limits. To give an example: liquid sugar cannot be used to sweeten packaged biscuits, bonbons, malted drinks, etc. Up to a certain percentage the syrup may be very useful in households as well, since it may enable the housewives to save time or even to introduce new dishes.

The manufacture of products made with liquid sugar requires a technology different from that used with granulated sugar. The new technology necessitates a minor adjustment in the course of manufacturing. This adjustment is simple and requires only a small, quickly returned investment. The United States of America, which up to now has covered nearly 50% of its sugar consumption from imports, intends to developed its liquid invert sugar production sufficiently to make imports unnecessary. At present 20—30% of the total sugar consumption is covered by liquid sugar. The fol-

lowing data are cited from the article "Fructose Producers Profitable with the 15 c. Sugar increase output" published in the Sugarbeet Grower, February 1975.

Sugar resources of the United States

in short tons:	1973	in the future
Domestic sugar-beet	3,512,000	4,000,000
Domestic sugar cane	1,527,000	2,200,000
Hawaii	1,142,000	1,200,000
Puerto Rico	79,000	200,000
Imports	5,330,000	—
Maize syrup	—	5,000,000
Total:	11,590,000	12,600,000

The production of liquid invert sugar in the United States is thus not a temporary solution but a process which is gaining ground, and which is less influenced by fluctuations in the world market price of sugar, the more so because there is little likelihood that the world market price of sugar will again sink to the low level of 1966.

In the United States, with a medium average yield, the above volume can be covered from 6% of the total maize yield, in such a manner that a good quality cattle feed is obtained as a by-product of the manufacturing process. I think that this trend towards stabilization will be established in Hungary too, to some 30% of the total sugar consumption. But this is not the only aspect; in the United States this type of sugar is believed to be healthier, being more readily converted by the organism. Thus it is a factor of civilized nutrition and represents a change in eating habits.

SÓLYOM, L.: The liquid invert sugar manufactured from maize means the appearance of a new type of sugar on the world market which cannot be regarded as a provisional solution.

SZENDREY, I.: Since the world market prices of hydrocarbons have shown an increasing tendency, and this increase has proved irreversible, the interest of the chemical industry in agricultural and sylvicultural raw materials has grown. Even those technologies which had ceased to be competitive with the old low oil prices are now being renewed, e.g. the hydrolysis of polysaccahrides by which sugar, then alcohol can be obtained. By eliminating the hydrocarbons, many important products can be obtained from alcohol, as a chemical raw material.

The appearance of the chemical industry as a big consumer of sugar has created a permanent sugar shortage on the world market. The United States has vast maize supplies, so the production of invert sugar from maize was found to be the most obvious solution of easing the sugar shortage. Starch is thus the polysaccharide hydrolyzed here. In the Soviet Union, on the other hand, the cellulose and hemicelluloses provided by the vast expanses of forest are hydrolyzed. This branch of industry progressed steadily here even at the time of the low oil prices. Industrial alcohol, fodder yeast and furfural are the main products today, but during the years of the two world wars sugar suitable for human consumption was the main product obtained from the hydrolysate.

The increased interest in invert sugar production from maize is a natural consequence of the radical change in the industrial raw material situation. It marks the beginning of a new era in which production technologies discovered long ago but pushed into the background during the period of cheap oil will be modernized and intensified so as to become competitive again.

The other main driving force behind the process is the increasing demand of the world population for food. In the new raw material situation the price increase of sugar, this basic foodstuff, can only be prevented by a substantial increase in production, for which the output of the traditional sugar-beet and sugar cane production is not sufficient. Polysaccharide sources such as the starch content of maize, though somewhat less readily utilizable, must also be tapped.

TARJÁN, R.: Prior to giving my opinion about manufacturing sugar from beet or maize I think it right to briefly mention the heated debate among nutritionists on the role in nutrition of crystallized carbohydrates (mono- and disaccharides), and their neutral or harmful nature. Some reasearchers consider the increased consumption of crystallized carbo-

hydrates during recent decades, which is still on the increase, to be decidedly harmful to health. A large number of researchers attribute some of the diseases peculiar to civilized communities (arteriosclerosis, diabetes, coronary diseases, etc.) to an increased sugar consumption.

Another large groupof nutritionists, while considering the increasing consumption of crystallized carbohydrates undesirable, feel that the arguments presented by the former group do not prove the harmful effects of these substances unequivocally.

Finally, a very small group of nutritionists are of the opinion that sugar consumption itself and an increase in the consumption rate while not perhaps favourable is at most neutral, though from the point of view of nutrition biology and physiology it would be better to keep the rate stable, or if possible to decrease it.

With our present knowledge it is very difficult to forecast the future of a cultivated plant, and as a nutritionist and physician I do not feel qualified to undertake the task (every respect to those who do so). In my opinion the invert sugar obtained during of maize will play an important role in human nutrition. To predict, however, whether it is maize or sugar-beet that will provide an overwhelming proportion of sugar production a wider and more thorough knowledge of the plant breeding, agrotechnical and food technological trends of the coming years would be required.

Considering the present deep-rooted feeding habits I think that granulated sugar will be needed, especially in households, for a long time ahead.

TYIHÁK, E.: The fact that the rediscovery of invert sugar production from maize coincided with the technico-scientific revolution and not the industrial revolution largely determines its future prospects. It is well-known that discoveries (rediscoveries) are now put into practice within a much shorter time than they were centuries, or even decades ago. It is also a fact that due to the faster rate of development each discovery (rediscovery) produces further discoveries, and development is definitely progressive. In my opinion the production of invert sugar will make a permanent conquest, but the earlier technologies will be replaced by modern methods of progressing, primarily those which ensure the complex processing of maize (invert sugar + protein + other materials).

VUKOV, K.: The manufacture of invert sugar from maize starch will be complementary to saccharose production for a long time to come.

ZELLER, GY.: I think that liquid invert sugar should certainly be regarded as a new product rather than as some kind of temporary solution to counterbalance market and price manoeuvres. In the case of new products it is important to decide whether their discovery or development promotes the fulfilment or better fulfilment of consumer demands; this must also be taken into consideration for liquid invert sugar. It is possible, and in fact usual for market conditions to accelerate or slow down the development of new products. The increase in demand, including the demand for a wider range of products, may be regarded, however, as an objective process. The question is whether the marketing of liquid invert sugar is an integral part of this process, or a solution adopted under the pressure of circumstances. If an answer is to be given to this question special attention should be paid to two facts. First, in the United States invert sugar has been produced on a large scale since 1974. The research, the experimental work, and then the investment activities preceding industrial production obviously took several years. Thus the decision to develop this product must have been made at about the beginning of the seventies, when a sugar deficiency or a rise in sugar prices on the world market were out of the question.

The other important point is that an unexpected shortage on the market can usually only be compensated by another product inferior to the missing one, since the rapid appearance and possible temporary character of the shortage will urge the producers to find some immediate transitional solution rather than to start a lengthy process of research and investment. Taking these and other aspects into consideration I am sure that liquid invert sugar is not a temporary solution but a new product to widen the choice, even if its introduction has been accelerated by negative changes in world market conditions.

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PÁL, GY.: **The production and processing of sugar-beet resulted in the transformation of European agriculture, as it involved more intensive farming methods, supplied a larger volume o**

fodder, wider employment, and also ensured more diversified nutrition for the population. Is it only the present labour shortage and the high labour intensity of sugar-beet production that justify the manufacture of invert sugar from maize, or will this remain important once the production of sugar-beet, like that of maize, is fully mechanized?

ALMÁSI, E.: Owing to the climatic conditions in Hungary considerable yield losses in certain crops have to be reckoned with from time to time. The sugar supply will be more reliable if it is based not exclusively on sugar-beet.

BAJNÓGEL, J.: The idea of producing invert sugar from maize and putting the sugar thus obtained on the market will probably still be viable in the future even if the technology of sugar-beet production is fully mechanized just as the technology of maize production is. The "competition" between the two types of sugar production will be decided by the specific data of crop production and sugar extraction, and by the production costs distributed over the whole vertical system. Of course, this may be so from the point of view of crop growing and sugar production, but may not be so clear where the consumers' attitude is concerned. In my opinion this is still a completely open question.

BARDACH, S.: In my opinion the industrial processing of maize is the main objective. One way of doing this, at present perhaps the most useful solution, is to compensate for the temporary shortage of beet sugar. Apart from this, however, there are many other possible ways of processing, which may be of even greater economic importance, including, naturally, those in which sugars are produced together with other products.

In acccordance with this the future of sugar manufacturing using maize as the raw material will be determined primarily by the future development of industrial processing, rather than by "competition" with beet sugar.

BENDE, P.: The fact that maize is easily stored and is therefore available all year as a basic material for sugar production may mean the survival of liquid sugar production from maize even when sugar-beet production is fully mechanized.

BODNÁR, M.: The full mechanization of sugar-beet production and the general application of an advanced technology can reasonably be expected to take place in the near future. In spite of this the chances of producing invert sugar from maize will not in fact be restricted, as sugar consumption is expected to increase dynamically in the future, particularly in the undeveloped countries (owing to the significant increase in the population growth rate primarily the total volume consumed, but parallel to this the specific (per capita) consumption too). Sugar consumption is definitely on the increase, though not at a very fast rate, in Hungary too, mainly in the form of sweets, chocolate, and other confectionery products, which are produced in ever greater variety by the confectionery industry.

BOLGÁR, P.: Although sugar-beet provides more favourable results expressed as yield per ha, still, owing to the lower production costs and specific investment requirements sugar-manufacturing from maize will always be competitive in Hungary.

FÜREDI, J.: The labour intensive character of sugar-beet production has resulted in a rise in sugar prices, which in turn has had a stimulative effect on the development of invert sugar production from maize. However, its future importance after the wide introduction of a mechanized technology for sugar-beet production depends on several factors, among others on the costs of raw material production and processing, as well as on the value of by-products (mainly as fodder) obtained in the course of the technological processes.

GALLÓ, GY : I do not think that the growing area of sugar-beet will increase even if sugar-beet production can be mechanized in the same way as maize production. The value of sugar-beet is decreased not only by its labour-intensive nature but also by its seasonal character.

GÁSPÁR, L.: If the production of invert sugar from maize is considered as an independent industry turning out a single end-product it is seen to be highly dependent on the consumer's taste, so in a necessarily conservative food industry its future position would be greatly influenced by the price trends of beet and cane sugar.

To a considerable extent the profitability of invert sugar production from maize can, however, be made independent of the fluctuations in the world market prices for cane and beet sugar, if maize sugar production is considered as one branch of a complex, vertically organized maize processing industry which produces a wide range of end-products. A maize industry of this type would produce invert sugar, starch, alcohol, oil, feed protein and pharmaceutical basic materials. This diversity, which could be further increased by introducing food products less well known in Hungary, would represent a high degree of stability for an industry where all the products obtained from a single raw material are valuable goods. Whereas in beet sugar production there is a substantial difference in value between the main product and the by-products, when manufacturing invert sugar from maize the differences are only quantitative; certain by-products, e.g. germ oil, may be more valuable than the invert sugar syrup itself. Nor must it be forgotten that, considering the present level of sugar consumption and the nutrition habits in Hungary, only a slight increase can be expected in the total sugar consumption, while the ratio of household to industrial sugar consumption will shift in favour of the latter, due to the increased consumption of tinned food. Invert sugar syrup will be used primarily by industry, as its use here involves technologic— and economic advantages.

On the basis of the above I do not think it probable, even with the expected development, that sugar-beet cultivation and beet sugar production will compete with a properly dimensioned maize industry, only intended to cover sugar requirements with sugar produced from maize where its use is really economical.

GYŐRVÁRI, I.: The full mechanization of sugar-beet production cannot make the justification of cheap invert sugar production questionable. Even the full mechanization of sugar-costs of technical development are ignored, though these are naturally not negligible from the point of view of the cost and income conditions in this field, the necessity of transporting about 5 million tons of beet containing 13—15% sugar, the seasonal character of the processing industry, and the substantial difference in sugar yield per unit area still remain to be reckoned with.

HOLLÓ, J.: Sugar beet, unlike maize, has been developed in European agriculture solely for the purpose of sugar production. To whatever extent the production of sugar from maize may develop in the future, the amount of corn used for this purpose will never be more than a negligible fraction of the total maize production. In spite of this, the introduction of maize as a supplement for sugar beet seems justified, as it is a specifically cheaper raw material with specifically low investment costs. These advantages outweigh the benefits of beet production, in spite of the fact that the latter offers higher yields with higher profits per unit land.

HORVÁTH, GY.: It is not so much the labour shortage, nor the present labour-intensive character of sugar-beet production, but rather other economic factors that primarily decide this question.

During the last few years, with the exception of 1976, the annual sugar consumption in Hungary was 460—480 thousand tons, 310—320 thousand tons of which were produced at home, while the rest came from imports. The reason why the domestic production was only 310—320 thousand tons was that the capacity was only slightly larger than this during a normal season. The replacement of imports, which is now one of the main concerns in Hungary, should be facilitated by the Kaba Sugar Factory and the Szabadegyháza Maize Sugar Factory.

The specific investment costs and specific operation costs are more favourable in the case of the maize sugar factory, so the manufacture of invert sugar from maize will remain important even if sugar-beet, like maize, is grown with a fully mechanized technology.

KISFALVI, T.: The processing industry investment per 1 ton of sugar is many times more for sugar-beet than when the raw material is maize.

Calculations show that in Hungary at present it is cheaper to produce sugar from maize.

The transportation of sugar-beet consumes enormous transport capacities at peak times, which are very expensive to maintain, while their utilization in other fields would save development costs at a national economic level.

Under our given energy conditions we cannot afford to heat large volumes of beet and water in order to extract 10—12% sugar content, when from maize some 60% sugar can be obtained using less energy and combined with more valuable twin-products. In practice the production of sugar from maize would mean the reduction in the import of energy carriers.

All these factors would be changed very little by the mechanization of sugar-beet production. It is another question how long our present and future sugar-beet processing factories can be rationally operated. The question will be settled by comparative calculations.

LŐRINCZ, J.: The mechanization of sugar-beet production has made great progress. In a few years' time sugar-beet will be grown at a mechanization level similar to that of maize production. To increase the digestion, however, means a more difficult task for breeder, producer and manufacturer alike. The new modern sugar factory and the eleven modernized factories are able to process the yield of the sugar-beet area, which is expected to become constant at 100—100,000 ha, in 80—100 days, so in my opinion they will be necessary in the future too.

NAGYPATAKI, I.: If we make a joint examination of the costs of raw material production and industrial processing we find that today maize is a much cheaper raw material for sugar production than sugar-beet. At the same production cost a higher value of useful dry matter per unit arable land can be obtained with maize than with sugar-beet, naturally taking into consideration not only the carbohydrate which can be transformed into sugar, but also the valuable protein and oil contents.

It is important to note that the value of the useful matter (carbohydrate, protein, oil) found in agricultural raw materials depends not only on its quantity but also on its concentration in the raw material. It is a well-known fact that shelled maize contains 81% useful dry matter (carbohydrate, protein, oil), while in sugar-beet, if the ballast materials (water, fibre, ash) are removed, only 15—16% useful dry matter is found.

On this basis it can easily be seen that maize, which has more than 60% starch content capable of decomposition to sugar, is a substantially more concentrated raw material for sugar production than sugar-beet. Therefore the same quantity of sugar can be obtained from maize in a much smaller processing plant. The specific investment costs and the fixed asset costs of operation are radically improved by the fact that a sugar factory using maize as raw material can be operated for more than 300 days a year due to the storageability of maize, while beet processing factories have an optimum operational period of 100 days a year.

In our opinion the deciding factor in the competition between the two raw materials is that for the nearly 32 million tons of beet sugar produced in the world annually some 300 million tons of sugar-beet have to be grown, transported and processed, while if maize is used the same result can be attained with 51 million tons of raw material, not to mention the much more valuable by-products obtained in the course of maize processing.

The much lower raw material transportation costs due to differences in volume and transportation distance must also be put to the credit of maize.

Sugar-beet is also unable to compete with maize in the sense that the latter, when subjected to processing, can be utilized in a great many ways, to produce dozens of end-products for human consumption and industrial use, compared to the one-sided and therefore limited utilization of sugar-beet. It is particularly noteworthy, however, that the water requirement, effluent production, assets, energy and labour demand per unit end-product are substantially higher in sugar-beet than in maize processing. At the same time, the range of end-products obtained from sugar-beet is very limited compared to the choice of product yielded by maize processing (including the most diversified sugar products).

Thus, if sugar factories based on maize are set up in the future, this step will radically improve the efficiency of agricultural production (change from sugar-beet to maize), transportation and industrial processing due to decreased labour costs, lower unit investment costs and reduced energy demand.

Basically this is also true of a comparison between maize and sugar cane, the other important plant supplying saccharose.

In those regions of the world where sugar cane is grown maize can also be grown or introduced. On some plantations maize is already substituted for sugar cane on the

consideration that the crop will mature in six months compared to the 18 months' vegetation period of sugar cane. Even if maize is harvested only twice from the same area during the vegetation period of sugar cane, the saccharose content of the grain yield will be at least, equal to that obtained from sugar cane under optimum conditions, In addition, maize contains not only carbohydrate but also considerable amounts of protein and oil. In other words, maize contains more nutritive matter, and is thus of greater value than sugar cane.

The introduction of new varieties and hybrids, together with mechanization, chemical plant protection, up-to-date soil cultivation and artificial fertilization have greatly increased the maize yields while maintaining a favourable level of prime production costs. At the same time, the composition of the maize kernel offers man the most important and most valuable nutrients in a fairly concentrated form, while the structure of the kernel enables the relatively easy extraction and separation of the different components (carbohydrate, oil, protein).

In addition, these advantages of maize and the results of this development are combined in maize growing with up-to-date methods of maize processing, including enzyme chemistry, by which maize can be converted into many kinds of food and industrial products as well as providing valuable protein fodder at favourable costs.

The possibilities of maize production and utilization discovered so far have provided an abundant source of food and raw material for the world population in a period when the increased production of important food stuffs is more and more needed.

It is quite natural that the increasing sugar demand of the world should be satisfied in the future by using maize as raw material, not only because such factories can be constructed and operated at lower costs than the beet sugar factories but also because by the decomposition of starch many kinds of sugar can be produced, meeting the most diversified special demands, which cannot be said either of sugar-beet or of sugar cane.

NÉMETH, S.: The use of maize grain for invert sugar production will remain important even if sugar-beet production is mechanized at a level similar to that of maize.

Comparing the yields of maize grain and sugar-beet root in Hungary I think that, under identical production conditions, approximately the same amount of sugar can be obtained per unit growing area.

At present 3—4 times as much manual work and time is required to produce the basic material for a unit amount of sugar from sugar-beet production as from maize production. With further mechanization the difference in labour utilization between the two crops will diminish but never quite disappear.

From an operative point of view the development of sugar production using maize as basic material is more favourable if only because in the autumn peak period of harvesting and transportation it is easier to organize harvesting and transportation for 60—80 q/ha maize grain than for 320—400 q/ha sugar-beet root. Particularly in the case of a rainy autumn it is much more difficult to organize undisturbed harvesting for sugar-beet than for maize.

One great advantage which will accrue to the national economy if maize is used for sugar production is that in the course of its industrial processing by-products can be obtained which, when processed, yield useful products for human consumption (maize oil rich in vitamin A), animal feeding and pharmaceutical purposes (antibiotics). On the other hand, Hungarian farms do not pay sufficient attention to the utilization of sugar-beet by-products, so a considerable proportion of them is lost or wasted.

PAIS, I.: It is not likely that invert sugar production will ever make the traditional sugar industry superfluous; the two technologies will be of use to man side by side, complementing each other.

PÁSZTOR, K.: At present with sugar-beet only a seasonal sugar production is possible, which means that there are labour, transportation and other difficulties. For this reason, even if the production of sugar-beet were fully mechanized it is not certain that, due to the above-mentioned restrictions, this would result in a change sufficient to render the manufacture of invert sugar from maize superfluous. It must not, however, be forgotten that the increase in the area and yield of sugar-beet is influenced by other factors too (limited availability of land, doubling of yield averages in a short time).

PECZNIK, J.—MAJER, J.: Sugar-beet production is already carried out at quite a high level of mechanization, and the development plans aim at full mechanization. In spite of this, sugar manufacturing from maize may be advantageous from the point of view of the national economy for the following reasons:

— From 1 q sugar-beet 12 kg, but from 1 q maize, by wet extraction, 50—60 kg sugar can be obtained. Thus, in the course of processing maize a much smaller amount of raw material has to be handled. The water requirement is also much lower. Since the dry matter content of the gritty fraction obtained on processing maize is about 30% compared to the 13% dry matter content of the diffusion juice obtained during beet processing, much smaller amounts of water have to be evaporated during the manufacturing process.

— For sugar-beet processing approximately 100 days, and for maize processing at least 300 days are available a year.

— There are very great differences in storage losses between the two crops. In 1975, for example, 4.5 million tons beet, containing about 15% sugar, were grown on 123.4 thousand hectares, which means that 675 thousand tons of sugar were produced in the field. From this the factories obtained only 460 thousand tons of white product. About half the 215 thousand ton difference was due to heading and storage losses, while the other half remained in the molasses and beet slices. In the case of maize the storage losses are an order of magnitude lower.

— During the industrial processing of maize, in addition to invert sugar, valuable products (protein feed, oil, corn steep liquor) can be obtained, which are readily sold on foreign markets, or may reduced the need for foreign imports.

POZSÁR, B. I.: The costs of sugar-beet production are expected to rise, and this will have a modifying effect on the prices. The demand for saccharose will survive even after the introduction of cheaper invert sugar, owing to conservativism in nutrition habits.

ROMÁNY, P.: The production and storage of maize are fully mechanized processes. As far as production is concerned, it is probable that sooner or later the same will be true of sugar-beet, but no cheap solution is ever likely to be found to the problem of preservation. Sugar manufacturing from maize is thus urged by many factors other than the present labour shortage and the high labour intensity of sugar-beet cultivation. To give a slightly distant but perhaps appropriate example: the Diesel engine was not invented because traction work could no longer be carried out whith steam engines, but because technical development made it possible to take a further step towards increased efficiency, and it would have been a pity not to take this step. The technology of beet sugar production is well known, and can undoubtedly still be improved to some extent, even though substantial efficiency reserves are no longer seen in it.

Invert sugar could also have been manufactured from starch non-enzymatically, and some attempts were in fact made in this direction, but like so many other technologies, this too is made much simpler and more economical by the use of enzymes. The enzyme technology, like progressive technologies in general, is becoming cheaper and cheaper. Let us go back to the analogy of the steam engine and the Diesel engine: the stimulus to development in other industries which was exercised by the appearance of the Diesel engine (in tool engineering, vehicle electricity, etc.) is widely known. The enzyme technology is expected to exert the same effect on the food industry.

RUFF, J.: The elaboration of production systems for sugar-beet similar to those in maize production is now in progress; they are expected to result in a substantial increase in yield and a reduction in the labour input.

In spite of this the importance of progressing maize into invert sugar will not change, since

— maize contains starch, the polymer of glucose, in a higher concentration than sugar-beet does, so its processing involves less machine capacity;

— owing to the concentrated nature of the raw material the demand for transport capacity substantially decreases (being only 20—25% of that for sugar-beet);

— due to its easy storability maize can be processed all the year round, whereas sugar-beet must be processed in a hurry.

These factors result in a significant reduction in costs. In certain areas of use liquid invert sugar might be highly competitive with cane and beet sugar.

SHMILLIÁR, M.: The favourable effect of sugar-beet production on the structure of farm management still holds true. Sugar-beet is a dual purpose crop, as its by-products, the leafy

head, slice and molasses, provide as much feed as a medium fodder yield obtained from an area of the same size. Moreover, the leafy head is also a noteworthy protein source, so much so that the protein deficiency shown in the fodder balance could be made up for by the yield of the present growing area of approx. 130,000 ha. It is another matter that only a small proportion of this protein source is utilized as yet.

As regards the labour intensity of sugar-beet cultivation, this is no longer a problem of much importance, since with the most recent technologies the manual labour input per unit area is only a tenth of what it was. Sugar-beet cultivation is now fully mechanized and the producer can choose the most suitable of a variety of machines and techniques. Beet sugar plays an important role in nutrition, but can in many cases be economically replaced by sweeteners produced from other raw materials.

The temporary decrease in the sugar-beet areas below the required level can be attributed to the joint effect of numerous factors. The first cause was the fact that Hungary's economic policy was based on an insufficient amount of information and was not progressive. The primary aim of the price policy which this prescribed should have been to give impetus to production, but this aim could not be attained, since the price policy was out of date. Market research, which was inadequate in this field, did not give due notice of the prospective trends in world market prices. We could continue to list the causes responsible for the opinion that Hungarian sugar production is no longer competitive on the world market, that the production costs are not in proportion to the established price, and that the sugar surpluses can be sold only at a loss, so that the whole question deserves little attention and still less development work. In fact these faults have now been eliminated and production has been stabilized, taking into consideration the demand and the possibilities. I emphasize that sugar-beet cultivation is only economical on certain areas, the availability of which is limited. The good maize growing area is many times larger. Sugar-beet cultivation will remain important, but the rapidly increasing demand for sugar justifies the manufacture of invert sugar from maize, all the more so because the latter can be more flexibly adjusted to a possible fluctuation in demand.

SÓLYOM, L.: Simultaneously with the manufacture of invert sugar beet sugar will maintain its leading roles as regards both industrial and individual consumers. The position of beet sugar is expected to improve in consequence of the full mechanization of sugar-beet production. At the same time, the position of liquid sugar in Hungary will be improved by the fact that the Hungarian sugar industry will be unable to satisfy the domestic demand in spite of the planned development.

SZENDREY, I.: Provided sugar-beet cultivation is successfully mechanized the production cost of beet sugar can be expected to decrease. This will make the alcohol produced from sugar still more competitive with hydrocarbons as a basic material of the chemical industry. So a possible increase in the production of beet sugar will not lessen the importance of invert sugar production from maize, as the demand for sugar will increase owing to a shift in the price ratios at the expense of hydrocarbons.

TARJÁN, R.: The manufacture of beet sugar transformed not only European agriculture but also the food industry, and at the same time had an influence on livestock keeping by providing a new feed base. The decrease in sugar-beet production and processing is thus only partly an agricultural and plant production problem and the consequences to the food industry and feed suppliers ought to be thoroughly analysed.

VUKOV, K.: Sugar-beet production can be mechanized very well, and is already mechanized to a great extent in Hungary as elsewhere. The greatest problem facing the producing farms is the transportation of the large volume of beet roots and tops.

Since sugar-beet production is largely mechanized in Hungary, the extent of invert sugar production will be determined by the production and transportation capacities.

ZELLER, GY.: In my opinion the full mechanization of sugar-beet production will not decrease the importance of liquid invert sugar manufacturing; its importance does not depend on the technology of sugar-beet production but on how the users and consumers judge this new product. Increasing consumption can be satisfied in two ways, namely:

— by increasing the production volume of existing products,
— or by covering the increasing requirements with new products.

There is a third solution: import; in our case, however, we are concerned with reducing imports.

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PÁL, GY.: **Until recently the sowing area of sugar-beet in Hungary decreased from year to year, while the maize area remained largely the same. In your opinion will the manufacture of invert sugar from maize cause a further decrease in the sowing area of sugar-beet, or will it leave the sugar-beet area unaffected and satisfy the growing household consumption of sugar by excluding the large industries from the consumption of beet sugar?**

ALMÁSI, E.: According to the current long-range predictions, covering the period up to 1955 (OMFB 8—7400-T, A kukorica ipari feldolgozásának fejlesztési kérdései (Development problems of industrial maize processing)) the production of liquid sugar will be 90 thousand tons a year. This will only cover part of the increase in sugar consumption expected between 1975 and 1995; in other words, it will not even cover the increase in household consumption.

BAJNÓGEL, J.: Supposing that a demand for invert sugar can be aroused, and that the producers have a sufficient financial interest in the production of both sugar-beet and maize, it will be the demand for beet sugar or invert sugar rather than the fact that invert sugar is being produced which will influence the sowing area of sugar-beet. As far as the demand is concerned, the possibilities of foreign trade must also be reckoned with.

It is likely that at the beginning invert sugar will mostly be used by the big consumers, rather than by households. This naturally means that for the big consumers invert sugar will partly replace beet sugar, which can then be used to satisfy the increasing demands of the households.

BARDACH, S.: Even in the developed industrial countries sugar products manufactured from maize have only been widely used for the last 6—7 years. This period is too short to assess the effect on beet sugar production. In spite of this I am prepared to risk the assumption that in Hungary the manufacture of invert sugar will not influence the sowing area of sugar-beet.

True, liquid sugar produced from maize will divert some of the industrial consumers from the use of beet sugar. However, judging by the available literary information, this proportion is not likely to exceed 20—25% of the total sugar consumption, which in Hungary means about 100,000 tons a year. Considering that the first factory to be built will produce 45 thousand tons of liquid sugar a year after 1980, liquid sugar production is unlikely to do more than stabilize the sugar-beet area.

BENDE, P.: In Hungary the consumption of beet sugar is usual and wide-spread. By comparison other sweeteners are only used in negligible quantities. In my opinion the sowing area of sugar-beet will not decrease any further in Hungary, and beet sugar production may increase in proportion to the increase in yield attained as the technology is developed.

In my opinion the production of liquid sugar is only one of the possible ways of utilizing maize as an industrial raw material.

It is mainly because of deep-rooted habits that in household consumption sugar-beet will not easily be replaced by liquid sugar produced from maize.

BODNÁR, M.: I agree with the opinion that the introduction of commercial invert sugar production will not essentially modify the present sugar-beet area in Hungary. It only means that the rapidly increasing demands of the industrial consumers will be met primarily with invert sugar, while the sugar demands of the households will be met mostly with beet sugar.

BOLGÁR, P.: When the new beet sugar factory goes into operation at Kaba a proper balance will be set up in the production and processing of sugar-beet in Hungary. This balance must be maintained, and new investments in this field must be directed towards using maize as a raw material.

FÜREDI, J.: Provided the present problems of sugar-beet production are solved, the manufacture of invert sugar is unlikely to cause a further decrease in the sowing area of sugar-beet. However, as an alternative means of obtaining sugar it may urge the solution of the present problems of sugar-beet cultivation. It must not be forgotten, however,

that the acreage of land suitable for sugar-beet production is limited, so its proportion compared to the maize area can hardly be increased. For big industrial consumers, provided the price is right, invert sugar may thus be a strong rival for beet sugar in the future, without, however, pushing it out of production, just as cane sugar has not been eliminated by beet sugar.

GALLÓ, GY.: Under the present conditions of mechanization and with the present yield averages sugar-beet production will slowly decline. As I see it, the big industrial consumers will change over to sugar produced from maize.

GÁBOR, M.: At least to start with no high demand for liquid invert sugar can be reckoned with, because of consumption habits. And if we consider the volume of production, no essential change in the sowing area can be expected in the initial period.

GÁSPÁR, L.: According to 1976 statistics the present annual sugar consumption in Hungary is 40 kg per capita. In my estimation (exact data in this respect are not known to me) 65% of this is consumed by the households and the catering industry; in other words, at present it need not or could not be replaced by liquid invert sugar. The remaining 35%, some 140 thousand tons, can be replaced by liquid invert sugar syrup, which, according to rough calculations, can be produced from 250 thousand tons of maize, that is, from less than 5% of the Hungarian maize crop. Considering the expected increase in average maize yields, this amount can be produced without extending the sowing area. A higher demand for raw material would only be raised if alcohol production from cereals for the food and chemical industries or for the extension of the power basis were to be substantially increased. This question is not closely concerned with the subject under discussion, but in connection with the maize industry it is worth mentioning that the demand for alcohol as an industrial raw material is steadily increasing, so apart from alcohol production from sugar-beet molasses and by organic synthesis the question of grain alcohol production also deserves attention. This industry, which has almost ceased to exist in Hungary, has a long tradition. It may be interesting from the point of view of agricultural industrial history to mention here that in 1870 at Martonvásár, now one of the centres of Hungarian maize breeding, Zólyomi-Wagner, professor at the technical university, carried out experiments on maize distillation in the local distillery, and due to an operational fault discovered an economical way of exploitation (Osztrovszky, Mezőgazdasági Ipar, Egyetemi Nyomda, Budapest, 1944, 266 p.).

GYŐRVÁRI, I.: I think that the sowing area of sugar-beet can only be decreased in proportion to the increase in yield averages. Invert sugar will certainly replace beet sugar only in industrial consumption, in Hungary as elsewhere. For household consumption conservatism will prevent the use of any substitute for beet sugar for some time to come. On the other hand, the per capita sugar consumption of 30 kg or so in Hungary does not represent a saturation level, which would mean a consumption of 45—50 kg sugar per person per year. Thus, industrial invert sugar consumption does not threaten the existence of beet sugar even if sugar-beet production is extended, since the per capita sugar consumption has increased by 1 kg a year for the last fifteen to twenty years. If the consumption continues to increase at this rate it will take another 10—15 years to reach saturation, which, according to our present knowledge, could not be covered from domestic beet sugar production.

HOLLÓ, J.: The sowing area of sugar beet in Hungary must not be reduced until the production per hectare and the sugar content have reached Central European levels. The 11 sugar factories operating in Hungary today, including the new sugar factory in Kaba, can only process the amount of sugar beet currently grown in a period longer than is economical. The present capacity of the beet-sugar industry does not cover the current requirements of the country. The production of sugar from maize might relieve some of our sugar import problems.

However, an increased production of maize sugar higher than the target amount, just because of its novelty, does not seem justified. Incidentally, the term "maize sugar" is somewhat ambiguous, if we consider the Hungarian term "potato sugar", which is, in fact, a mixture of glucose and oligomers prepared from potatoes by acid hydrolysis. The product to be prepared now is glucose, fructose (and not invert sugar), with a minimum amount of disaccharides caused by the incomplete decomposition of starch.

HORVÁTH, GY.: The manufacture of invert sugar from maize will not reduce the sowing area os sugar-beet, as the latter is determined by the yield averages of sugar-beet.

The sugar-beet processing capacity of the sugar factories is a constant. Therefore changes in the sugar-beet yields determine the length of the season in the sugar factories.

It is obvious that when the Kaba Sugar Factory goes into operation the sowing area of sugar-beet is likely to increase. Invert sugar manufacturing will only divert a very small proportion of the large industrial consumers from beet sugar consumption. At present the initial capacity of the liquid invert sugar factory has been fixed at 45,000 tons a year. Once the invert sugar production is in full operation an equivalent amount of beet sugar, intented mainly for large industrial consumers, will no longer be imported.

During the last two years the sugar production, consumption and import have shown the following trends in Hungary (according to rounded data):

		1975	*1976*
beet sugar production	1000 tons	320	370
sugar consumption	1000 tons	480	420
sugar import	1000 tons	170	150

Of the sugar imported in 1976 a considerable volume (some 100 thousand tons) was left over for 1977.

KISFALVI, T.: What has been said before cannot be left out of consideration when examining this question either. It should be kept in mind that our exports have been in the order of a million tons for several years, but if processing facilities were available the processed products (sugar, alcohol, starch, concentrated protein feed, germ oil for human consumption) would be easily marketable, and would mean a surplus foreign exchange in the order of a hundred million dollars (calculated for a million tons of maize grain raw material).

Therefore the formulation of the question is not complete; export aspects should also be taken into consideration when analysing the problem and forming an opinion. With adequate information and advertising invert sugar produced from maize may overcome the aversion to anything new and unusual just as the different kinds of cola, which are undoubtedly startling when first tasted, have been accepted for consumption. The household consumption of invert sugar, in a thick, honey-like state, can and must be reckoned with. It would be a pity to waste energy on concentration, as in most cases liquid state sugar satisfies the requirements of household consumption.

This question too, with the addition of the above information, will be decided after comparative calculations based on the productivity and beet supply of the existing sugar factories, the rate of development of the maize processing industry now under construction, and the domestic and foreign market trends, using an optimum calculation series.

LŐRINCZ, J.: Provided the large-scale experiences of invert sugar production are both technically and economically favourable, part of the ever increasing sugar demand could well be satisfied with invert sugar produced from maize. A more urgent task now is to prevent the reduction in the sowing area of maize due to the increasing fodder demand for livestock. Since sugar-beet production at a level of 280—350 q/ha (which can be reliably maintained) is economically favourable and fits in well with the production structure, I do not expect the sowing area of sugar-beet to decrease in the coming period, even if the production of invert sugar yields favourable results.

NAGYPATAKI, I.: Of course, initially the increasing sugar demands will be covered from maize, so the new invert sugar factories will necessarily be established in maize growing regions, after the model of the development of maize processing during the last few years in the United States of America.

Later the gradually depreciated beet and cane sugar factories will also be replaced by factories using maize as raw material, so the production of sugar-beet and sugar cane will gradually lose its importance and the sowing areas will decrease. Accordingly, in the regions concerned the sowing structure will fundamentally change, though not at a spectacular rate. This development trend was initiated in order to

save labour and development resources, and to satisfy the demand for important but unavailable foodstuffs, and the process will be hastened by the protein deficiency and by the production of protein fodder obtained as a by-product of maize processing.

NÉMETH, S.: The manufacture of invert sugar from maize will result in a certain reduction of the sowing area of sugar-beet. According to my estimations it will decrease by about 25—30%.

PAIS, I.: Under the influence of the new technology the sowing area of sugar-beet will probably decrease, though to a greater extent only after 8—10 years.

PÁSZTOR, K.: The manufacture of invert sugar does not at present affect the sowing area of sugar-beet. If the yield averages of maize increase at a faster rate than those of sugar-beet it is likely to lead to a decrease in the sugar-beet area. A feed shortage could cut down the volume of maize used for invert sugar production. When comparing the two crops the energy output value must also be taken into consideration.

PECZNIK, J.—MAJER, J.: In our opinion, taking prospective yield increases into consideration, the final sowing area of sugar-beet will be about 100 thousand ha. This will not be essentially modified by the industrial processing of maize. Maize, or the glucose produced from it, may be used as a basic material for sorbite and ascorbic acid production, for example. Sorbite is used in considerable quantities in diabetic and other food products. Ascorbic acid (vitamin C) is used by the food industry in ever increasing amounts as an antioxidant, for vitamin enrichment, and for consistency improvement (in the baking industry).

POZSÁR, B. I.: When invert sugar is put on the market it will primarily satisfy the requirements of the confectionery industry. In private households, despite the lower price, its substitution for saccharose will be a slow process. I think that once sugar-beet production has been fully mechanized both the sowing area and the sugar content per unit dry matter will increase.

RIGÓ, J.: From the point of view of nutrition biology the sugar consumption in Hungary has exceeded the desirable level. While in the 1930s it was about 11 kg, by 1975 it reached an average of 39—40 kg per capita. Any further growth in this more than threefold increase would be unfavourable for health. A further increase in the sugar consumption would promote the consumption of superfluous, extra calories. However, within the framework of sugar production an internal re-structuring is conceivable, but only in a form that excludes a further increase in the total volume.

Knowing the nutrition habits in Hungary, I do not think it will be possible to substitute invert sugar for the kinds of sugar (granulated sugar, icing sugar, etc.) used in the households. Its use in considerable volumes can be expected primarily in distilleries, confectioneries and in the canning industry. It is known what technological adjustments will be necessary for its use, and economies in the utilization of beet sugar can be expected primarily from the industries. A switch-over to the new technology makes it possible to modify the earlier standards according to the biological requirements, and to put products with lower sugar contents on the market.

ROMÁNY, P.: Initially, invert sugar manufacture will only satisfy the growing sugar consumption of households in an indirect way, by channelling beet sugar away from large industrial consumers into retail trade. A further substantial decrease in the growing area of sugar-beet is not expected.

RUFF, J.: In Hungary the production of liquid invert sugar in the near future is designed to lessen the shortage of sugar, mainly in those fields where the traditional beet sugar is in any case subjected to inversion before use, or where fixed price food products call for the use of a cheaper raw material.

Besides all this, some increase in the sugar consumption of households must also be reckoned with, although they will mostly continue to use beet sugar.

SHMILLIÁR, M.: On the basis of what has been said above, the reduction in the sowing area of sugar-beet was a natural process. The comparatively small farms were not sufficiently mechanized and were thus hard hit by the decrease in the available labour force. In

the larger farms, on the other hand, there was a disinclination to assume the amount of risk necessary in all sectors of production, and convenience became the dominant factor. Wheat is undoubtedly easier to produce economically than sugar-beet. In addition, many people feel it would be better to import cheap sugar. In order to eliminate these factors producers now benefit from various price subsides, which make them more interested in production. The regulation of the purchase prices has ensured the economic efficiency of production.

The extreme weather conditions in recent years, the rapid increase of the production area from 70 to 130 thousand ha, the incorrect application of different fertilizers, particularly nitrogen, and the mistakes inevitably made when changing over to a new technology resulted in a lower sugar content in the beet compared to earlier years. Therefore the reduction of the sugar-beet area back to the earlier acreage, on which the yield averages did not develop satisfactorily, was still unable to supply sufficient sugar for Hungary. The demands are constantly increasing. A certain degree of sugar shortage has occurred again. The manufacture of invert sugar from maize is intended to play a certain role in satisfying domestic sugar consumption, to make up for shortages which may occur due to failures of the sugar-beet crop, and to provide raw material for certain products. At the same time, its by-products will furnish valuable feedstuffs.

SÓLYOM, L.: The introduction of invert sugar manufacturing must not be allowed to influence the sowing area of sugar-beet, as Hungary makes up for the deficiency of its domestic sugar supply by importing sugar.

SZÁNTÓ, S.: The sowing area of sugar-beet will in all certainty decrease in the future, if liquid invert sugar suitable for industrial consumption is introduced. This is all the more probable as the national per capita average sugar consumption in Hungary is close to 40 kg/year, and according to the opinion of nutritionists any further increase would be undesirable even from a nutritional point of view.

SZENDREY, I.: The fact that the surplus demand has become permanent is the justification for the production of invert sugar from maize. This product can replace beet sugar for all uses where its different character does not represent any particular disadvantage in the course of further processing. This does not mean that with the utilization of the new source of sugar the sowing area of sugar-beet can be further reduced in Hungary. Invert sugar is an ally rather than a rival to beet sugar in satisfying the increased and manifold demands.

VUKOV, K.: The sowing area of sugar-beet in Hungary increased from 70,000 ha in 1971 to about 125,000 ha by 1975. The volume of sugar produced cannot, however, keep abreast of the increasing domestic consumption, since owing to the limited processing capacity larger sugar-beet crops are processed at increasing losses. The most significant of these losses is that occurring in the sugar content during the storage of beet.

Special arrangements must be made for the storage, transportation and reception of liquid invert sugar. Aseptic conditions are required for storage over a longer period, so it is expected to be used mainly in larger factories.

ZELLER, GY.: I think it is reasonable to cover the increased demand with liquid invert sugar, primarily by cutting down the beet sugar consumption of the industrial consumers, thereby enabling the growing household requirements to be fulfilled from an unchanged sowing area of sugar-beet.

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PÁL, GY.: The large industrial sugar consumers (confectionery and liqueur factories, preserving factories) and even households utilize granulated beet sugar mostly in a dissolved state. Considering, however, that habit is of great importance in the case of commercial goods, do you think that a comparatively higher consumption of liquid invert sugar by households or the food industry can be expected, or that, for want of state subsidies, it may perhaps be used for adjusting the sugar percentage of grape juice?

ALMÁSI, E.: Only a fraction of the invert sugar (a few percent) is consumed by households. Naturally, it can also be used to adjust the refraction value of grape juice.

BAJNÓGEL, J.: This problem is closely related with those which have gone before. I think that in the initial period of invert sugar production, and probably later too, most, if not all of the demand for liquid invert sugar will come from the big industrial consumers. An important role is played in this by the conservativism of the consumers, but other aspects, e.g. kitchen technology, are also important.

Before answering this question two very important points have to be taken into consideration. First, the per capita sugar consumption (including both sugar in its natural state and industrially processed products) is expected to reach saturation level at about 46 kg, and then to slightly decrease, while an increasing proportion of the sugar will be consumed in the form of industrially processed products (of the confectionery, preserving and catering industries). Secondly, the big industrial consumers are technically better equipped to receive liquid sugar, and with suitable production technology can make more rational use of it for their products than the households.

BARDACH, S.: Liquid sugar will be used almost exclusively by industrial consumers. Its consumption by the population cannot be reckoned with because of the cost-increasing effect of retail sale packaging and transportation, not to mention the conservatism of the housewives.

BENDE, P.: Liquid sugar obtained from maize will be substituted for beet sugar by the large industrial users and will therefore have a positive influence on the sugar balance in Hungary.

I consider the use of liquid sugar to adjust the refraction value of grape juice to be a make-shift, as is the use of beet sugar for this purpose.

BODNÁR, M.: It is true that crystallized beet sugar is mostly used in a dissolved state by both industries and households. Yet a distinction should be made between the large industrial consumers and the households, which cling to habits, traditions and prejudices. It is probable that the big consumers will accept and use invert sugar at once, while the households, although they also show an interest in it, will only partially and gradually get used to the consumption of invert sugar. To promote this a regular educational programme should be set up on health matters and on proper nutrition, and this should be intensified in the near future. In my opinion a state subvention should be granted for the production and marketing of invert sugar, at least in the initial phase of mass production.

BOLGÁR, P.: Owing to its physical state, the liquid sugar produced from maize will, for a long time, only be important for industrial consumers, including the wine industry, of course.

FÜREDI, J.: Larger volumes of liquid invert sugar are undoubtedly expected to be consumed primarily by the liqueur, preserving and confectionery factories, where sugar is used in aqueous solution. But there is nothing to prevent its being used for household consumption too. This will be first and foremost a question of marketing technology and price, and the two types of sugar may well be used side by side.

GALLÓ, GY.: The big industrial consumers will switch over to the use of liquid sugar, as it is easier to handle and saves a work process. Households are bound by traditions to a greater extent. The extent to which the users are equipped for the reception and storage of liquid sugar is an important question. Current practice requires a dry storeroom, where a fairly large stock can be stored. The change-over is facilitated by the punctuality and flexibility of transportation.

GÁBOR, M.: The sweetness-degree and the character of the sweet flavour of invert sugar and saccharose solution are different at the same concentration. In addition the physical properties of the two materials are different in a dissolved condition (e.g. owing to the presence of fructose the invert sugar solution is hygroscopic, furthermore it has an inhibitory effect on crystallization). These differences will initially determine both household consumption and the industrial utilization of invert sugar.

GÁSPÁR, L.: In my opinion liquid sugar will play no part in household consumption, as this would mean returning to the practices of the 18th century or earlier, when cane sugar syrup and honey were used as sweetening agents.

The treatment of grape juice with invert sugar syrup instead of beet sugar syrup, although it would be an improvement, is not a question of wine technology but a matter of legal regulation, of permission or prohibition.

GYŐRVÁRI, I.: A larger volume of invert sugar consumption in the households can probably be expected only with a substantial, 40—50% price difference in favour of invert sugar, otherwise it will mostly be utilized by industry.

HOLLÓ, J.: In some western countries, wholesale consignments of beet sugar are now delivered in the form of liquid sugar, owing to the problem of re-dissolution. In our domestic trade, the present consumers of the new type of sugar are large industrial concerns, but it is possible that in the future the product may also be made available to the retail trade, pre-packed in small amounts for household consumption. In theory, both types of sugar are suitable for increasing the amount of sugar in grape juice, but the prohibitions prescribed by the wine law are valid for all types of sugar.

HORVÁTH, GY.: In human and animal organisms the simple sugars and polysaccharides partly serve the purpose of producing energy (glycolysis), and partly accumulate in the organism as nutrient reserves (glycogen-glycogenolysis). All functions, however, require the decomposition of di- and polysaccharides to monosaccharides as a basic step, since human and animal organisms are not able to utilize them in any other form.

When beet sugar is used as a constituent of food products, the organic acids contained in fruits, and even the CO_2 found in fizzy drinks, invert the beet sugar into glucose and fructose.

When added to grape juice or wine beet sugar is also inverted under the influence of the organic acids present. In the liqueur industry beet sugar is inverted by adding organic acid (e.g. citric acid, tartaric acid) to it before use, and the same is done in soft drinks factories, canneries and sweet factories. In the German Federal Republic and the Scandinavian countries artificial honey — invert sugar made from beet sugar and flavoured with honey aroma — is marketed for food purposes. It may be clearly seen from the above that the beet sugar used in the food industry is returned to the consumer in the form of invert sugar (except for wineries and breweries, where fermentation products are mostly produced). In spite of this, liquid invert sugar cannot be purchased for household use either in the United States or in Europe, so in Hungary too it will be used primarily in food processing factories.

It will, of course, be suitable for adjusting the sugar percentage of grape juice. In wine making crystalline dextrose or even glucose molasses have also been used to strengthen the grape juice. It is thus obvious that liquid invert sugar, a much more refined product than the former, must also be suitable for this purpose.

As regards the invert sugar requirements of the food industry, the following information has been obtained so far:

	annual requirement in dry matter tons
Brewery Trust	22,000
Winery Trust	9,100
Distilleries	10,000
Budapest Mineral Water and Ice Enterprise	6,300
	47,400 ton dry matter/year
Preserving industry (estimated)	22,000 ton dry matter/year
Other enterprises (estimated)	13,000 ton dry matter/year

The total demand is far more than the amount of maize invert sugar Hungary will be able to produce.

KATONA, J.: In all grape growing countries the wine law prescribes the materials to be used and the methods of processing. In Hungary, unlike many other countries, the wine law does not permit the use of beet sugar for the amelioration of grape juice and wine. In 1975 the International Office of Viti- and Viniculture began to develop a uniform world policy of viti- and viniculture. One of its objectives, aimed chiefly at decreasing overproduction, is to replace beet sugar by sugar produced from grape juice. In those coun-

tries where the use of beet sugar has been permitted so far, this practice is expected to change.

Aromatized wines, in which the use of beet sugar is permitted, are produced in fairly large volumes in the world. In my opinion the use of liquid invert sugar instead of beet sugar can only be reckoned with for these products of wine making, and even then only under the following conditions: they must not contain harmful components, they should be harmless to health, they must not impair the organoleptic properties of the wine, nor interfere with easy handling, and last but not least, its use must be licensed by the wine law.

KÁDÁR, GY.: The necessity of permitting the use of sugar in wine making has caused heated discussions. At present there is no legal provision to make the general use of invert sugar in wineries possible.

The decree No. 25/1970/XI. 26.) MÉM issued by the Minister of Food and Agriculture, in other words, the enacting clause of the wine law, declares that the use of sugar or sugar solution is only permitted in making fermented sparkling wines and vermouths. The production of both kinds of drink, but particularly that of sparkling wine, is increasing at a fast rate (at present the yearly fermentation amounts to about 20 million bottles). The sugar requirement of these drinks is not insignificant. The use of invert sugar solution is undoubtedly more favourable than that of crystallized beet sugar. The wineries, like other branches of the food industry, will certainly welcome the invert sugar solution.

The addition of invert sugar to grape juice in order to raise its sugar content will be unavoidable in the future, according to the opinion of many experts. However, the suggestion that the necessary sugar content should be brought about in the grape itself by the proper choice of site and variety and by rational agrotechnics is steadily gaining support. This is not a difficulty which is impossible to overcome, but our large-scale vineyards and the plant protection service face a considerable task (more frequent change of variety, rational nutrition, efficient control of Botrytis, etc.) if the above objective is to be realized consistently from year to year.

In the future the production of soft drinks, an industry consuming an extremely large volume of sugar, must be increasingly reckoned with. This industry has made amazing progress in recent years. While in 1960, for example, the per capita consumption of soft drinks in Hungary was hardly 5 litres, by 1970 it had reached 13.6 litres, and by 1975 34 litres. Thus, in 1975 some 10% of Hungary's total sugar production was consumed by the soft drinks industry. The production of fizzy drinks shows an extremely dynamic growth. On this basis the per capita consumption is expected to reach 54—55 litres by 1980, which means that this industry will use about the same amount of sugar as the confection industry. And for 1990 a soft drinks production of 95 litres per capita is estimated, so that this industry will be the largest sugar consumer of all the branches of the food industry. This dynamically developing line will obviously welcome liquid invert sugar.

KISFALVI, T.: The answer given to the former question is essentially relevant here, with the added information that the glucose produced during maize processing can be used in wine making.

LÁSZTITY, R.: Industrial consumers will probably take the lead in utilizing liquid sugar. According to the estimates the industrial demand will not be fully satisfied for quite a long time. As regards household consumption the role of habit is much greater. Apart from this, the possibilities and potential advantages of liquid sugar utilization are also less distinct.

LŐRINCZ, J.: The extensive utilization of invert sugar has great possibilities in the food and wine industries. Preparations should be made, however, for the transportation and storage of the liquid raw material.

NAGYPATAKI, I.: The advantages of liquid sugar can best be exploited by the food industry and confectioneries to improve the characteristics of their products, since it is cheaper than beet sugar, as well as labour-saving. Large volumes of liquid sugar are expected to be consumed primarily by the canning and confectionery industries and in the manufacture of beverages. In countries where it is not against the law it can also be utilized with advantage to increase the sugar percentage of grape juice.

For household consumption liquid sugar can be marketed, like honey, in bottles or jars, either in its natural form or flavoured according to consumer demand.

As conservative consumption habits are most dominant in the households, the use of liquid sugar will spread more slowly here than in industry.

Population demand will probably be stimulated in the developed countries by the production of liquid sugar in a form containg at least 90% fructose, as this kind of sugar is sweeter than the usual beet sugar. The desired sweetness of foods and drinks can thus be attained with smaller quantities of sugar, which means a saving for the household and a lower calory consumption for the human organism.

High fructose liquid sugar can accordingly be consumed by diabetics, as well as by those requiring a specially mild diet.

Through the decomposition of maize starch not only can liquid sugar of various sweetness, containing dextrose and fructose in different proportions, be produced, but also icing sugar and granulated or cube sugar.

NÉMETH, S.: In my opinion the food industry will consume a relatively larger volume of liquid invert sugar than the households.

PAIS, I.: Habit is undoubtedly an important factor, but if the consumers are satisfied with the new product it does not make any essential difference in some fields of household consumption whether the sugar is used in a solid or liquid form.

PÁSZTOR, K.: Confectioners, preserving factories, pharmaceutical works, etc. can utilize liquid invert sugar. It could theoretically be used to raise the sugar percentage of grape juice, but at present the wine law only permits concentrated grape juice. I doubt whether the use of liquid sugar will spread very quickly in the households, except seasonally for bottling fruit, although the present housing conditions do not render it possible to store bottled fruit for very long.

PECZNIK, J.—MAJER, J.: Liquid invert sugar is expected to be used in quite large volumes in the food industry, where it may be a great advantage that the storage and handling of sugar in a liquid state can be fully mechanized. If the dry matter content of the invert sugar is about 70%, the fluid can easily be pumped, and there is no danger of fermentation, so storage losses can be practically eliminated. Invert sugar is more suitable than saccharose for adjusting the sugar percentage of grape juice, provided this is not against the provisions of the wine law, since the latter cannot ferment until inversion has taken place.

POZSÁR, B. I.: I expect the demand of the confectionery industry to cause an approximately 30% replacement of saccharose in industry and some 10% in the households, provided invert sugar can be put on the market at a substantially lower price.

ROMÁNY, P.: The different branches of the food industry will be the first consumers of liquid invert sugar. I think, however, that consumption by the population must also be reckoned with. In the United States a considerable percentage of the liquid sugar is consumed by households, and relevant technical journals forecast a further increase in household consumption. In the retail trade in invert sugar the packaging technology represents the greatest problem, but as shown by the various kinds of sugar flacons seen on the shelves of American food markets this problem can be solved.

As for the utilization of sugar in wine-making, this is controlled by the wine laws.

RUFF, J.: The main users of liquid invert sugar, as I have already mentioned, will be big industrial consumers, since.

— the labour- and energy-intensive dissolving procedure will become unnecessary,

— the probably favourable price (since there are no crystallization, drying and packaging costs) will substantially reduce the prime cost of the product,

— it is easier to transport and store.

SHMILLIÁR, M.: Referring once again to an American example, a Canadian company (Canada Dry Corp.) uses fructose in all its fruit juice and soft drink products without ever having observed any negative effects. The Coca-Cola Company uses fructose in all its fruit juices and intends to use it in manufacturing Minute Maid lemonades as well. Habit

and adherence to tradition with respect to the introduction of liquid invert sugar are only likely to be evident in the households. But even there I do not think the objection will be unsurmountable. Modern consumers are not averse to trying new products, and will continue to use them if they prove to be good. Not so long ago some people regarded machines as enemies, while today not only industry but households too aim at full mechanization. With proper informatory work, sensible advertising and clear instructions housewives can probably be convinced that in certain cases they can use invert sugar economically. In the United States the greatest problem at present is that the sugar is not available in sufficient quantities. The increased rate of nutrient replacement in vineyards ensures large yields. Large yields, however, almost invevitably involve a lower sugar content, not enough to ensure the required quality. In order to ensure a reliable yield, particularly as regards quantity, producers tend to gather the grapes earlier. This is also justified by the fact that diseases which occur during ripening and cause decay are difficult to control. The large yield and the early vintage render it necessary to raise the sugar level of the grape juice, which can be done most simply by adding sugar. Experiments should be carried out to find out whether liquid sugar can be used for this purpose. If so, its use should be permitted by the wine law. If properly applied this procedure could result in improved quality. Constant high quality is indispensable, as Hungarian quality wines are known and sought in almost every part of the world.

SÓLYOM, L.: Invert sugar is expected to penetrate all fields of industry where at present beet sugar is utilized. Industrial consumers are expected to be the main users, but the new product may gain ground in the households as well.

SZÁNTÓ, S.: The consumption of the food industry will exceed that of the households. In the households tradition will favour the consumption of granulated sugar. Consumption by the food industry will be influenced by the existence of liquid sugar receiving stations. If the ministry provides the financial means in time, industrial consumption will be continuous and uninterrupted.

TARJÁN, R.: In Hungary, as in all industrially developed countries, industrial sugar consumption represents a considerable and ever increasing proportion of the beet and cane sugar utilised. Before any further research and investigation is begun in the field of crystallized and invert sugar production it would be desirable and also necessary to carry out preliminary studies on food technology, in order to find out what technological adjustments (investments) should be effectuated by the present large food industry consumers (confectioners, baking industry, fruit preservation, etc.) before they can substitute invert sugar for the granulated sugar currently used.

Households insist at present — and in my opinion will do so for a long time ahead — on using crystallized sugar, particularly in the increasingly popular form of cube sugar. (I should like to refer briefly to how rapidly loaf-sugar, which was the most usual form of sugar 30—40 years ago, was replaced by granulated sugar, and then the latter by cube sugar.)

ZELLER, GY.: As I have already suggested, it is in the industrial utilization of liquid invert sugar that I see the main possibility of a rapid change in consumption patterns. This does not mean, of course, that liquid invert sugar is to be excluded from household consumption; however, the consumption habits of the population take much longer to change than the utilization habits of industrial users do. The right methods of sales promotion (attractive packaging, advertising and favourable prices) could lead to liquid invert sugar having a considerable share in household consumption within a few years.

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PÁL, GY.: **In Hungary the starch content, determined by fermentation, in a maize grain crop with a 12% water content is 56—60%; in the case of wet extraction 56—60 kg refined invert sugar can be produced from 1 q maize. In your opinion, are the current maize varieties and hybrids really suitable for invert sugar manufacturing? In the future will the varieties bred for protein and oil content be suitable for invert sugar making, or will new varieties with a high starch content have to be developed?**

ALMÁSI, E.: Target production should be introduced in maize growing too.

BARDACH, S.: Calculations show that the industrial processing of maize already decided upon is economical even using the varieties currently in commercial production.

There can be no doubt that the processing of special hybrids could be the source of further advantages. This possibility can only be realized, however, if the advantages and costs of the varieties are reconciled. I should like to call attention to the fact that initiatives aimed at extending the industrial processing of maize, mainly for the purpose of producing new feedstuffs, are now being worked out. If this development is to be successful the appearance in commercial production of economically cultivated varieties with components more favourable than those of the present varieties may be of decisive importance. This is one reason why the co-ordination of research carried out in various fields is so important for the economic utilization of maize as a raw material.

BENDE, P.: The maize varieties grown in Hungary mostly meet the requirements of invert sugar production. Taking into account the kernel components of the average maize crop, higher starch content varieties need not be developed for the production of liquid sugar by means of complex processing.

I think it extremely important, on the other hand, that the varieties grown should reach maturity by the time of harvesting, so that the nutrient reserves should be present in the grain in the form of starch.

BODNÁR, M.: Although the empirical data according to which 58—60 kg refined invert sugar can be obtained by wet extraction from 1 q maize grain and 11—12 kg refined sugar from 1 q sugar-beet are in fact correct, this does not mean that maize yields five times as much sugar as sugar-beet, as is shown quite clearly in a paper by Imre Nagypataki (Magyar Mezőgazdaság, Információk, No. 44, 29th October 1975).

According to my calculations, based on specific yield and production cost indices, which rely mostly on factual data (50 q maize/ha, 200 Ft/q maize; 380 q sugar-beet/ha, 55 Ft/q sugar-beet), 30 q/ha invert sugar can be obtained from maize, and about 45 q/ha beet sugar from sugar-beet. It is also true, however, that this 15 q/ha higher sugar yield is produced by sugar-beet farmers at twice the cost (20,000 Ft) of the 30 q/ha invert sugar yield produced by maize farmers. Apart from this, the savings due to the storability of the basic material for invert sugar production from maize compared with beet sugar production, and thus the distribution of the production process over the whole year, together with the lower specific energy costs as regards raw material transportation, mean in fact that the mass production of invert sugar from maize could be considerably cheaper and more economical than beet sugar production. As I see it, there is no need to develop new high starch maize varieties, even if varieties improved for protein and oil content come into prominence in the future, partly because the stabilization of Hungary's feed protein base is still regarded as a highly important central programme, which takes priority over sugar production, and partly because, however successful the breeders may be, the protein content of maize will never, due to biological and genetic factors be high enough to reduce the starch content significantly.

BOLGÁR, P.: In Hungary healthy maize produced commercially is suitable for sugar manufacturing. As an industrial raw material I do not think that it would be influenced very much by breeding in the short term.

FÜREDI, J.: I am of the opinion that most of the maize varieties and hybrids currently grown are suitable for invert sugar production, provided they are harvested at the right stage of ripening and with optimum water content, and stored until processing under appropriate conditions. Breeding for increased protein content naturally decreases the relative starch content, but at the same time the higher protein by-product obtained in the course of processing will be sold at a more favourable price.

Breeding for oil does not necessarily reduce the starch content, because the oil is not located in the endosperm. On the contrary, breeding for increased oil content may promote invert sugar production, if not directly through the structure, but indirectly through the spreading of maize suitable for oil production, since the endosperm, when the germ has been removed, will provide suitable raw material for the invert sugar factories. It is possible, however, that the extension of different types of industrial processing will result in the differentiation of maize varieties, as has occurred with other crops, according to the purpose of processing.

GALLÓ, GY.: The starch content and extractibility of maize varieties grown in Hungary do not satisfy the demands of production. Starch content is not the only qualitative determi-

nant. Extractibility has a considerable influence on the degree of utilization. The maize varieties with which I am familiar can only partly fulfil these requirements. The extent to which the starch is bound may vary a great deal. The high protein content is an inhibiting factor if the extractibility during the soaking period is not sufficient. The structure of the seed-coat also plays an essential role.

Varieties bred for protein and oil content influence the starch content unfavourably. From the point of view of starch extraction, medium late varieties are generally the best. Too early, small-grained maize varieties are not suitable for starch production. Late varieties are often immature. It seems desirable to develop maize varieties with a high thousand-grain-weight for this purpose.

GÁBOR, M.: From an economic point of view varieties with a relatively high starch content and lower protein and oil contents are the most suitable for invert sugar production. The presence of the two latter components influences the technology (possibly rendering it more complicated and expensive), since their disturbing effect, and consequently their removal must be reckoned with in the technological process.

GÁSPÁR, L.: In answering the question concerning the quality of the maize raw material I can rely on my own investigations. Results are available from several years' studies on the quality of Hungarian maize hybrids and of those originating from the major maize-producing countries of the world. Leaving the special botanical types and special endosperm mutants out of consideration, in normal dent corn and smooth-grained maizes only the variability of the three main qualitative components, starch, protein and oil content, is important. The mean value, minimum, maximum and variability of these factors are: starch content (polarimetrically): mean 63.9%, min. 58%, max. 68%. CV = 3.29. Protein content (Kjeldahl N × 6.25): mean 9.01%, min. 6.55%, max. 11.9%. CV = 13.96. Oil content (by Soxhlet extraction): mean 4.47%, min. 3.27%, max. 5.26%. CV = 13.62. There is a significant negative correlation between the starch and protein contents and a non-significant correlation of negative tendency between the starch and oil contents. Differences in crop-year and growing site are of the same order of magnitude as the variability obtained with different hybrids grown at the same site in the same year. It can be seen that the variation coefficient is the lowest for the starch content. Thus, from the point of view of chemical composition the variety is not of determinative character for industrial processing. The fact mentioned above that the by-products are also valuable does not mean that breeding for special requirements need not be continued.

We have no experience concerning the milling qualities of the different hybrids. The amount of starch which can be extracted and converted into sugar is also influenced by this property. In the United States there are special dry and wet methods of maize processing in the milling industry. It seems to be worth making a comparative evaluation of maize raw materials with the laboratory or semi-operative forms of these methods.

As a long-term aspect it may be mentioned that if a complex maize industry is to be set up the value of various endosperm mutants from the point of view of the starch, sugar and fermentation industries should be examined. I am thinking here of the waxy maizes; to the best of my knowledge amylopectine is in great demand and sells at a good price.

HOLLÓ, J.: The maize produced in Hungary for feeding purposes, provided it meets standard qualification requirements, is suitable for the production of the new sugar. Considering the estimated raw material requirements of the new sugar factory, as well as the development in maize and sugar production, the amount of maize currently produced in Hungary as a raw material resource for the new industry, is insignificant (1.5%). Depending on the technology, oil is separated from maize in the course of degermination, and soluble proteins are obtained and utilized at different technological levels; therefore maize varieties and hybrids grown for both protein and oil are suitable for sugar production. In my opinion, there is no need to breed varieties with a specially high starch content for this purpose, particularly as such varieties generally produce lower yields.

HORVÁTH, GY.: The raw material for maize starch factories in Hungary consists at present of hybrids containing 56—60% starch.

The dextrose produced in the Ászár starch factory is also made from this starch. Although the maize utilized meets the requirements of the Pécs and Ászár starch fac-

tories, it is very far from being perfectly fit for this purpose, and will be still less suitable for invert sugar production. It took a long time to develop the high starch hybrid American YML (yellow maize 1), in which, according to analytical data prepared by Matsui and Co. Ltd., the starch content exceeds 68% even on a wet basis (shelled May corn). Nor must we omit to mention that in the United States Vineyard and Bear reported as long ago as 1952 on hybrids in which the amylose component of the starch could be increased to 60% without any decrease in the total starch content; then in 1954 Deatherage and his collaborators reported on the development of a maize hybrid in which the total starch content increased, with a simultaneous increase in the amylose content to above 60%. But many cases of failure could also be mentioned, when the gene mutation was accompanied by adverse side-effects.

For example, in experiments aimed at increasing the proportion of essential amino acids, which resulted in the development of the Opaque-2 hybrid, Lambert et al. (1969) found that the amino acid composition of the maize protein satisfied the requirements, but that the yield fell by an average of 8%, the hundred-kernel-weight was 5% lower, the moisture content increased by 20%, the proportion of broken grains by 89% and the oil content by 13% compared to the counterpart hybrids.

However, researchers have also found Opaque-2 hybrids in which the above disadvantages do not appear, which suggests that in spite of initial failures the objectives of research on Opaque-2 hybrids have been realized; thus today the decisive economic requirement for Opaque-2 hybrids is a high lysine and triptophane content rather than a high yield.

Nelson et al. (1965) reported on the increased lysine and triptophane contents in Floury-2 hybrids, similar to those in Opaque-2.

In July 1975 the "Magyar Szó", a Hungarian newspaper published in Novi Sad (Yugoslavia), reported that N SSC 418 F, a hybrid maize with the most favourable amino acid composition in the world, had been developed by Dr. Rejla Savic, professor at Novi Sad University. In this hybrid the lysine content is the same, and the methionine content is three times greater than that of the Opaque-2 hybrid. These hybrids have been produced exclusively for feeding purposes.

In the United States the Illinois High Oil maize varieties have been developed by selection; they combine a high oil content with a favourable oil composition. These varieties serve for the production of maize oil.

It would thus be a great pity to use high protein hybrids with favourable amino acid compositions, or maize varieties with high oil contents for invert sugar production. For this purpose it is worth developing high starch and high amylose hybrids adapted to the Hungarian climatic conditions, even though the raw material supply for the starch and invert sugar industries will not exceed 2% of the domestic maize yield once the manufacture of invert sugar has started. (In the United States 10% of the maize crop is processed by the starch, corn syrup and food industries.)

Maize is a responsive plant, worthy of the attention of plant breeders. With proper attention the yield averages may be expected to increase so as to satisfy both the special demands of industry and the requirements of animal feeding.

KISFALVI, T.: The question of how profitable maize varieties bred for protein, oil or starch will be using different processing methods, from the point of view of the national economy, the producers and the processing enterprises, will be decided by their yield per ha, their price, the internal ratio of the three components and the market price of the processed products. A satisfactory answer, or answers, to the question can only be given once the above information has been obtained and optimum calculations have been made; an estimate will only ever be suboptimum.

LŐRINCZ, J.: Just as the extraction of a considerably larger amount of sugar than at present faces the sugar-beet breeders and producers and the processing industry with many new tasks, the maize breeders have to develop special hybrids for this purpose. One or two of the existing hybrids may be suitable for the economic production of invert sugar.

NAGYPATAKI, I.: The maize varieties currently employed for mass production in Hungary and in the developed maize growing areas of the world can all be used in starch factories for the production of liquid sugar or other starch derivatives.

Though some fluctuation in the ratio of carbohydrate, protein and oil may be observed as a function of variety and crop year, this is of little consequence as regards

the cost of production and the volume of products obtained from maize, provided healthy, undamaged maize kernels are used for processing.

Moreover, with the present techniques of maize starch production varieties with protein or oil contents substantially higher than usual can also be used. Of course, in this case the protein or oil yield will be larger and the amount of starch and sugar correspondingly smaller.

In this context a cause of constant misunderstanding should be mentioned. Although the amount of sugar obtained from maize is many times greater than that produced from the same volume of sugar-beet or sugar cane, it is impossible to regard maize simply as a raw material for sugar manufacturing, partly because it is a raw material which also contains protein and oil, and partly because not only sugar but starch, many starch derivatives and alcohol can also be produced from it, together with a valuable quantity of yeast and protein.

Protein and oil are also highly valuable components of maize, as it is not so much carbohydrates as proteins that are badly needed in human nutrition and animal feeding, while maize germ oil also plays an important role in nutrition.

In this sense it would be very misleading to simplify the comparison of sugar-beet and maize and to restrict the importance of maize to the extraction of sugar, because this importance lies, apart from the advantages mentioned, in the possibility of choosing the variety to be grown according to the required composition and in adjusting the complex utilization according to the current needs.

This possibility of complex utilization will ensure that in the maize processing factories to be constructed in the future the range of products will be composed according to the current market demands.

Is the coming years the accent in development is expected to be on sugar, as the favourable sugar prices and the rich choice of sugars adjusted to special demands will greatly stimulate liquid sugar consumption.

The production capacity of the first liquid sugar factory will not cover Hungarian requirements, so it will be necessary to construct further factories.

NÉMETH, S.: Although a large proportion of the hybrids commercially produced at present can be used for invert sugar production, the development of hybrids with high starch contents must be one of the future objectives of maize breeding. The setting of this task is also justified by the fact that starch content and productivity are in positive correlation.

PAIS, I.: Starch content will certainly be an important requirement, but since the greater part of the maize crop will remain a feedstuff for animals, the results achieved so far in breeding will not be wasted.

PÁSZTOR, K.: The present ma ize varieties can temporarily be used for invert sugar production but in the long run speci al hybrids with increased starch content and higher productiv ity will be needed. We must also think of hybrids whose straw could be economically used for invert sugar production. Dual-purpose hybrids with high oil and starch contents might also be a possibility.

PECZNIK, J.—MAJER, J.: As regards the properties of the maize to be processed the following points should be taken into consideration:

— If possible maize varieties should be used, or developed by breeding, in which the endosperm and the embryo can easily be separated from the grain. It is important that the endosperm should be practically free of lipid substances.

— If invert sugar is produced with a dry technique, efforts should be made to increase the proportion of the glassy (gritty) fraction. In the course of the relevant examinations it has been found that the glassy fraction in two of the maize hybrids grown in Hungary is about 50%, compared to the other varieties in which the glassy fraction is 35% or so.

— When choosing the variety attention should be paid to other products and by-products which can also be obtained from maize (starch, glucose, alcohol, oil, maize cake, bran, yeast, enzyme preparations, maize stillage, corn steep liquor, etc.). Apart from the technological aspects, detailed economic calculations may thus be of decisive importance here.

POZSÁR, B. I.: I do not think it likely that starch and oil type maize varieties will be developed in less than ten years. It is thus probable that the maize crop will be used in two ways, similarly to the industrial technology in the United States.

ROMÁNY, P.: The technology of invert sugar manufacturing is quite flexible and in the production process not only the starch content of the maize is utilized. Thus, an increased starch content in maize cannot be the only breeding aim. Accordingly, any decision on breeding maize for increased starch content could only be made in the light of knowledge of the cost structures in an already working factory. However, as I have said, the problem is not urgent, since the factory will process only a fraction of the total volume of maize produced in Hungary.

RUFF, J.: In the case of complex maize processing the present hybrids bred for protein and oil meet the requirements of invert sugar production, since the by-products are much sought after on the domestic and world market and thus make the processing profitable. Here I am thinking of germ oil, alcohol and protein-rich fodder. Hybrids with a high starch content are naturally desirable from the point of view of sugar production, as the above-mentioned valuable by-products disturb the processing to some extent; therefore, quite apart from economic considerations it is necessary to extract them from a technological point of view as well.

SHMILLIÁR, M.: The production of invert sugar from maize is most economical when using varieties with a high starch content. Such varieties should thus be grown on a certain percentage of the maize area, mainly in the neighbourhood of the future processing plants, so as to save expenditure. The importance of varieties improved for oil and protein content is not affected by the manufacture of liquid invert sugar. It may well be that maize oil will be produced in the same factory; the two are thus complementary to each other. Varieties improved for protein content play an important role in fodder mixtures.

In my opinion any maize variety or hybrid not specially improved for a certain purpose is suitable for liquid invert sugar production. Economic calculations are thus required to decide which varieties are worth using for invert sugar production. The possibility of three-way utilization, e.g. for oil, invert sugar and fodder production, should also be taken into consideration.

SÓLYOM, L.: As a result of the complex processing of maize different components are extracted at various cost prices in the course of invert sugar manufacturing. Considering the economic aspects and the planned maize processing capacity, it seems reasonable to choose varieties with higher starch and lower dissolved matter contents, the maturing time and harvesting conditions of which are the most favourable for manufacturing liquid invert sugar.

TYIHÁK, E.: The current varieties of maize meet the requirements, particularly those of complex processing.

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PÁL, GY.: **The amount of saccharose obtained from sugar-beet is determined by the volume of sugar-beet produced and by its sugar content; the grain crop of maize can be stored for an unlimited period, so the manufacture of invert sugar is not of a seasonal character, production can continue all the year round if the demand for invert sugar makes it necessary. After the completion of the planned invert sugar factory what do you think will determine the volume of invert sugar production: the production capacity or consumer demand?**

ALMÁSI, E.: This depends on the economic conditions. Even in the food industry, factories have to make preparations for the use of invert sugar. The problems of transportation and storage must be solved, and technological changes may also be required. If the price difference between invert sugar and beet sugar is large enough not only to cover all these expenses, but also to make the utilization of invert sugar economical, then after a certain trial period the volume of invert sugar production will be determined by the consumer demand.

BAJNÓGEL, J.: As to the volume of invert sugar to be produced I think that it is basically a matter of planning. In my opinion it would be desirable to carry out market research in order to estimate the prospective demands of the big consumers and how these are likely to increase in the future. The capacity of invert sugar production should be adjusted to the demand. Of course, the planning of the production capacity must not lose contact with the maize growing basis.

Since risks are involved in the introduction of the product on the market and the assessment of the demands, to start with a production capacity designed to cover but not exceed the expected demands should be envisaged.

BARDACH, S.: When planning the maize processing plant to be built at Szabadegyháza, the prospective demands of the potential industrial consumers were taken into consideration, on the basis of trial processing performed with imported liquid sugar. In the course of the survey claims were put in for nearly twice as much as the planned volume of production. Accordingly, after 1980 the quantity consumed will probably be determined by the production capacity. The technological limits to the industrial utilization of invert sugar can be outlined. When making further decisions concerning an increase in the capacity these claims, as well as the concrete demands arising when the first factory has been put in operation, must be taken as the basis.

BODNÁR, M.: The question of whether production capacity or consumer demand will determine the volume of invert sugar production once Hungary's invert sugar factory is put into operation depends on a number of factors. The primary role of production capacity depends, for example, on the dimensions of the factory or factories in question, while the trend of consumer demand is influenced mainly by two factors, namely:

— the success of the nation-wide nutrition biological education mentioned above, and

— a change in the price ratio of beet to invert sugar in favour of the latter.

If these two factors which influence consumer demand act in the same direction (additively), then the volume of invert sugar produced will be determined mainly by the increase in consumer demand.

BOLGÁR, P.: Once the maize-sugar factory goes into operation further investments of this kind will be determined by the consumer demand.

FÜREDI, J.: I think that besides production capacity and consumer demand the volume of invert sugar production will be essentially influenced by the price, as by the production, processing, marketing and other costs.

GALLÓ, GY.: The production in the planned invert sugar factory will be determined by the capacity. To start with, until the big industrial consumers have accepted the big industrial consumers have accepted the new product, there are likely to be difficulties in making full use of the capacity.

GÁSPÁR, L.: In my opinion the use of invert sugar will be determined by the production capacity. Demand may at best have an influence on the extension of the capacity.

GYŐRVÁRI, I.: Consumer demand.

HOLLÓ, J.: The development of domestic sugar production from maize will be determined by the sugar supply and demand at the time when the first such sugar factory is put into operation in Hungary.

HORVÁTH, GY.: Sugar-beet processing in sugar factories is, indeed, of a seasonal character, while the manufacture of invert sugar from maize is almost continuous. The planned number of workdays/year is 300, while the remaining 65 days are required for maintenance work, of which the August—September maintenance period will take up 45 successive days. During this period the users will rely on invert sugar reserves, or use beet sugar.

After the completion of the planned maize sugar factory the volume of invert sugar production will naturally be determined by the production capacity, and not by the consumer demand. The average quotation price for a giant plant producing 45,000 tons of invert sugar dry matter a year is nearly 18 million US dollars without assemblage, construction and know-how.

KISFALVI, T.: The capacity for invert sugar production is expected to be the limiting factor for a long time to come, as consumer demands are reported from both the domestic and international markets; in other words, there seem to be wide marketing possibilities for Hungary on the markets of developed and developing countries alike.

LŐRINCZ, J.: Since the consumer demand is determined by many factors, this question can only be answered after a complex analysis of the work of the invert sugar factory. In my opinion, if production is cheap and efficient, then the consumer demand can be increased together with the extension of the capacity of the factory. It is up to the manufacturers to widen the range of products made from invert sugar, so that consumption could be increased at the same time.

NAGYPATAKI, I.: Since it is a new product, certain reservations expressed in connection with the consumption of liquid sugar are quite understandable. For this reason it should be objectively stated that with regard to its origin (plant origin) and composition (a sugar mixture similar to honey) liquid sugar belongs to the natural system of carbohydrates which are well known to play a highly important role in the living world, while the techniques employed in liquid sugar production are adjusted to the strictest criteria of the food industry. The purity and ash content of the liquid sugar in question is more favourable (refined to a higher degree) than that of beet sugar.

In some countries including the United States of America, the marketing of liquid sugar and its use as a foodstuff were legally permitted some 1.5 million tons this year, so presumably the consumption of liquid sugar must be about the same. The biggest users of sugar in the world, which supply tens of millions of people every day, including among others the Coca-Cola company in the United States, have authorized the use of liquid sugar.

NÉMETH, S.: In the interests of the national economy, once the invert sugar factory is completed the volume of invert sugar produced must be determined by the production capacity, and the consumer demand must be influenced in this direction by means of economic incentives.

PAIS, I.: In my opinion consumer demand may be a significant factor at the beginning, while later the production capacity may act as a limiting factor.

PÁSZTOR, K.: Once the construction of the invert sugar factory has been completed, the production volume will depend on both the production capacity and the consumer demand. When considering the production capacity it must be remembered that the continuous production throughout the year enables quite a large volume of invert sugar to be manufactured. As far as consumption is concerned, foreign trade must also be taken into consideration.

PECZNIK, J.—MAJER, J.: We do not think it probable that the consumer demand will be less than the production capacity of the planned factory. In developing the production technology of the factory the following points have to be taken into consideration:

— Since the manufacturing is **not** seasonal, continuous operation enables the work to be organized economically.

— If the so-called wet technique is used, various kinds of sugar (refined, liquid invert sugar, granulated glucose, malt sugar) can be obtained.

— Syrups in liquid or pulverized (maltiron) form can be produced with sugar contents meeting the users' demands, broken down to various degrees by the hydrolysis of starch. These are absorbent, film forming, foam stabilizing materials of various sweetness and solubility, which depress the freezing point.

— Starch can be produced from maize, and dextrines from starch.

POZSÁR, B. I.: In my opinion the production capacity will be the limiting factor of utilization right from the beginning.

RIGÓ, J.: In my opinion the production of invert sugar will be determined by consumer demands, and it will take quite a long time for even the food industry to substitute invert sugar for beet sugar.

RUFF, J.: In Hungary the production level in the planned invert sugar factory will definitely be determined by the production capacity, because

— in the case of an initial lower demand for invert sugar, glucose can be obtained as an intermediate, which is also a product sought after on both the domestic and the world market;

— in the course of processing the proportions can be shifted towards the valuable by-products (alcohol, feed starch), for which hydrolysed starch is again the raw material.

It is clear from the above that even the most extreme market fluctuations can be abated by adjusting the products structure.

SHMILLIÁR, M.: The manufacture of liquid invert sugar from maize has the great advantage that it is not seasonal in character. The future factory should be constructed with a capacity sufficient to ensure undisturbed ,continuous year-round operation. The demand is expected to amount to 20—30% of the total sugar consumption. It will probably be exportable at a suitable price, so this must be taken into consideration when deciding on the capacity of the factory. The production of this commodity is increasing all over Europe. Negotiations are now taking place in Belgium concerning the construction of a factory. These arrangements suggest that the demand for this product is generally rising. The demand, as mentioned above, can in fact be influenced by means of appropriate advertising. If Hungary appears on the international market with this product in good time, there will be marketing possibilities as well.

SÓLYOM, L.: Once the construction of the invert sugar factory has been completed, the production volume will be determined by domestic and foreign consumer demand, as a function of the current position of beet sugar.

TYIHÁK, E.: This is largely a function of the prime cost.

VUKOV, K.: When the invert sugar factory under construction in Hungary goes into operation the volume of invert sugar to be produced will be determined by the consumer demand until the conditions for extensive utilization are provided.

ZELLER, GY.: In my opinion the full utilization of production capacity in the invert sugar factory now under construction will be attained in a relatively short time; this will depend mainly on the industrial consumers. It is thus obvious that for a short time the available production capacity will be the determining factor. The possibility of continuous production is a very great advantage, resulting in a high degree of utilization of the assets engaged. For the long term, however, the starting point must be the consumer demand, which, after a certain period, will probably exceed the available production capacity.

I think therefore that from the time the product is put on the market conditions must be continually and systematically studied; the prospective demands should be predicted, and the time when a balance is established between supply and demand, or the occurrence of excess demand should be determined; on the basis of all this a decision should then be made in good time on a possible increase in capacity. With a product like this, which is expected to have a long life on the market, it is essential that even a temporary supply problem should be avoided. It is an article of daily consumption, which means that it must be constantly available in a wide network of shops.

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PÁL, GY.: In the course of manufacturing invert sugar from maize a 1 : 1 mixture of glucose and fructose is produced at the third enzymatic stage. Owing to the presence of fructose this mixture is tastier and sweeter than glucose alone, but out of consideration for the elderly and sick do you not think that glucose ought to be regarded as an end product, or that for the sake of diabetics the process should be carried to the point of transformation into fructose?

ALMÁSI, E.: A low-capacity pilot plant could carry out market research to assess the demand for products containing a mixture of glucose and fructose or glucose only. In my opinion the household consumption will be negligible.

BAJNÓGEL, J.: A certain amount of fructose should be extracted from the invert sugar to be produced, as it will probably be requested. Naturally medical opinions are needed if it

is to be marketed. Insofar as the idea raised in the question agrees with professional opinions, suggestions might be made concerning the use of fructose in cooking and baking.

BARDACH, S.: Glucose is an intermediate product in the production of liquid sugar from maize. The technology used at Szabadegyháza renders it possible to remove some of the output in this state as an end product.

BODNÁR, M.: In my opinion the best solution would be to take into consideration the empirical and statistical data concerning old people, invalids and diabetics, and to separate the successive enzymatic stages in the process of manufacturing invert sugar in such a way as to obtain both glucose and fructose in adequate proportions.

BOLGÁR, P.: The production of pure fructose has no economic basis as yet.

GALLÓ, GY.: The ratio between the two components, as well as further processing, will be decided be the demands of industry.

GÁBOR, M.: With a view to making production economical and the product utilizable, and taking the relatively low demand by diabetics into consideration I think it would be expedient to omit the enzymatic transformation into invert sugar. The enzyme procedure might be inserted to a minor extent in the case of diabetic products.

GÁSPÁR, L.: The problem of producing sugars, glucose and fructose, for pharmaceutical purposes must be kept separate from the development of the maize sugar industry. As far as I know the number of cardiac cases and diabetics is on the increase, but fortunately not sufficiently to justify the establishment of a new industry. Invert sugar production is aimed at covering, totally or partially, the sugar requirements of the food industry by means of a product conforming with the traditional consumer's taste, and within the framework of a complex maize processing industry. This does not mean, however, that, should it prove necessary and economical, the sugar requirements of the pharmaceutical industry cannot be covered from this raw material basis.

HOLLÓ, J.: The Hungarian starch industry is already producing hydrolysis products with very varied DE (Dextrose-Equivalent) values ranging from starch syrup to pure glucose (dextrose injection). But there is no reason why pure fructose should not be produced for diabetics using the enzymatic technology in the newly established factory units. This is, in fact, only a financial problem, as the latter procedure involves much higher expenses.

KISFALVI, T.: The technological problems involved in producing different kinds of sugar can be solved, and it would be desirable to adjust the ratio of the products to the current market demands. In this respect the industrial processing of maize provides a highly advantageous technological background.

LŐRINCZ, J.: Invert sugar will be the main product. The ratio of the different products will be decided by economic calculations.

PÁSZTOR, K.: According to my knowledge, fructose is not good for diabetics either.

PECZNIK, J.—MAJER, J.: The nutritive value and fermentability of glucose can be considered identical to the corresponding properties of invert sugar. However, since it is much less sweet than the latter, glucose is less suitable for sweetening purposes. The fructose tolerance of diabetics is not substantially higher than their invert sugar tolerance. In our opinion it seems reasonable to regard invert sugar as the main product, though this does not exclude the production of dextrose and fructose. In actual fact consumer demand and economic calculations will decide the volumes and proportions of the different products.

POZSÁR, B. I.: It is highly probable that glucose could also be a food product suitable for commercial utilization. At the same time, fructose ought to be produced from inulin (artichoke tuber).

RIGÓ, J.: The question deals with the dietotherapic aspect of the end-product of the decomposition process. Both glucose and fructose are primarily sources of calories. In a pure state they are therefore used mainly in roborating diets as calory sources promoting the incorporation of proteins. This is particularly important when the patient is fed with quickly absorbed, shorter or longer carbon cycle peptides or amino acids. Elderly and sick people generally require mainly body building and protective nutrients, as their demand for calories is lower. Thus, neither glucose nor fructose has any special role in dietotherapy. Experiments performed with animals convincingly prove that infarctoid myocardial lesions are mostly promoted by fructose. This is confirmed by our own experiments, carried out with honey. No protective effect can thus be expected from fructose.

Several decades ago diabetics were given glucose (in Hungary in the form of artichoke syrup), because free fructose is decomposed in a somewhat different way than glucose. However, fructose, in the form of dihydroxy-acetone-phosphate may ultimately enter the process of glycolysis, the Embden—Meyerhof pathway, directly and may thus play practically the same role as glucose in the glycolysis process.

I should recommend invert sugar primarily as a nutrient for sportsmen and heavy manual workers.

ROMÁNY, P.: The technology of the factory under construction renders all this possible, but it would not be reasonable to readjust the production of a large factory on each occasion just to fulfil such relatively low-volume demands. On a national level the demand for glucose is not significant and can be covered in the future by expending the starch manufacturing capacity. The utilization and production of fructose for direct consumption is not expected in the near future.

SHMILLIÁR, M.: The production of fructose has always been proportional to the number of elderly people and diabetics. If this proportion increases, as it is doing at present, this must, of course, be taken into consideration. The increase in life expectancy also affects this subject, but from the point of view of total consumption the actual growth of the world population is of greater importance, since this will be doubled within a relatively short time. Living standards in the developing countries are steadily rising, and this coincides with an increasing sugar consumption. This enormous mass of people are all prospective sugar consumers. I think it probable that when the liquid sugar factory goes into operation it will produce both types of sugar in a volume sufficient to cover the requirements.

SÓLYOM, L.: Glucose is already manufactured in Hungary and is commercially available under the name "Corvital". Considering the expected demand for fructose by diabetics it does not seem economical at present to transform part of the liquid sugar produced into pure fructose. The problems of diabetics should be solved in other ways.

SZÁNTÓ, S.: It is intended that liquid invert sugar should replace saccharose wherever this is possible. From the point of view of nutrition biology the two kinds of sugar fall into almost the same, not very favourable category.

TARJÁN, R.: From the point of view of nutrition biology even the present level of crystallized sugar consumption is unfavourably high. Any further rise would be undesirable. However, we have to reckon with the fact that, owing to the high pleasure value represented by sweets, and by foods and drinks made with sugar, radical changes in our feeding habits cannot be expected.

Sugar with the highest possible sweetening capacity should be put into circulation, because in that way the consumption of crystallized carbohydrates may be reduced while the pleasure value remains the same. With this in view it is important to increase the proportion of fructose in the mixture of monosaccharides as much as possible. Fructose is absorbed in the organism at a slower rate, is converted via a metabolic pathway different from that of glucose, and affects the insulin-producing mechanism of the organism to a lesser extent. These facts justify the transformation of glucose to fructose. In the case of diabetics, for instance a daily fructose surplus of 50 g is generally permitted by the doctors in addition to the given consumption of carbohydrates.

TYIHÁK, E.: Since the technology is adjustable, both products can be produced according to need.

VUKOV, K.: Invert sugar production from maize starch can be solved with a one-stage enzyme treatment on the basis of a Hungarian patent already tried and tested in a pilot plant.

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PÁL, GY.: **The invert sugar produced from maize grain can be crystallized in the same way as beet sugar and it dissolves in water just as well, so it can be produced in either the solid or the liquid state. Do you think liquid invert sugar will be accepted by the consumers, especially by households, as a commercial product, or will it have to be produced for them in a solid state, even if this involves extra expense?**

ALMÁSI, E.: For households liquid invert sugar may mean an extension of the choice. It is marketed in this way in other countries too.

BAJNÓGEL, J.: On the part of the big industrial consumers I do not think that any objection is likely to be made to the use of invert sugar in a liquid state. However, the crystallized form will be required for certain products (e.g. in the confectionery industry).

In my opinion very few, if any households will accept invert sugar. Some of the sugar is, of course consumed in the households in a dissolved state, but baking and cooking require crystallized sugar as well. Nor must it be forgotten that if sugar is bought in a liquid state housewives will have to carry 40—45% heavier packages home form the shop than when buying granulated sugar.

The sale of liquid invert sugar for household consumption would not be in the interests of the national economy either, since the packaging of liquid invert sugar for retail trade would require about 60% more storage space at all levels of commodity sale, and about the same proportion of extra transporting capacity, compared to granulated sugar. Naturally, the handling costs would also increase.

BARDACH, S.: In the present phase of development the crystallization of sugar produced from maize is not planned, mainly for energetic considerations.

BODNÁR, M.: Liquid invert sugar will certainly be accepted at once by large-scale consumers, since it is more advantageous for them, while for the households invert sugar should be marketed, at least to start with, in the form of granulated or cube sugar.

BOLGÁR, P.: The planned maize-sugar (a mixture of glucose and fructose) cannot be crystallized, so it would be better to leave household consumption out of consideration.

FÜREDI, J.: Invert sugar could well be marketed in both the solid and the liquid state.

GALLÓ, GY.: In satisfying household demands a proper ratio between liquid and granulated sugars should be maintained. There are cases where the use of liquid sugar in the household is almost impossible. These requirements must also be taken into consideration.

GÁBOR, M.: The production of invert sugar in a solid state, besides increasing the direct costs of investment and production, increases the packaging and storage problems, owing to the hygroscopic character of fructose.

In the retail trade, however, considering the demand and habits of household consumers, only the solid form of invert sugar can come into question. It is very difficult to use invert sugar syrup in households, if it can be used at all. To mention only one example: all cake recipes would have to be rewritten, not only because of the different composition of the sweeteners used, but because they also influence the physical and colloid structure of the cake mixture in other ways.

GÁSPÁR, L.: As I have mentioned before, I do not think there is any sense or future in trying to introduce liquid invert sugar for general consumption.

HOLLÓ, J.: Although the glucose-fructose syrup obtained from maize cannot be crystallized, it is just as soluble as beet sugar. This new product could be introduced and would be acceptable for household purposes, just like honey. However, as far as I can see, retail sale for household consumption would not coincide, for the time being, with the interests of the industry.

HORVÁTH, GY.: The invert sugar produced from maize in the form of a 71—72% solution is competitive with beet and cane sugars.

Since it would not be competitive in a granulated form, there is no plan to market it in this form for household consumption.

LŐRINCZ, J.: I think that invert sugar will mainly be consumed in the liquid state, since it is difficult to crystallize. Since the food industry and wine making require a liquid raw material in any case, it would be wasteful to insist on crystallization, which will almost certainly be expensive. In the case of households a honey-like product may perhaps be accepted and used. Once transportation and storage are solved large canteens, the army, etc. may also become consumers of considerable importance. However, it must be taken into consideration that the sweetening effect of invert sugar is 4—5% lower than that of beet sugar.

NÉMETH, S.: The handling and storage of liquid invert sugar will not cause any problem to industrial consumers, and from the point of view of work organization liquid goods are easier to handle. For households invert sugar must be produced in a solid state, even if this involves additional costs (owing to traditions, easier portioning and storage)

PAIS, I.: I do not think that the production of a solid form of invert sugar will ever be necessary.

PÁSZTOR, K.: Household consumers must be made accustomed to the use of "liquid sugar". If the former possibilities for making bottled fruit still existed, the use of liquid sugar for this purpose would be more favourable, but the sugar used to sweeten food is preferred in a solid state.

PECZNIK, J.—MAJER, J.: To the best of our knowledge invert sugar is less easily crystallized than saccharose. If for no other reason, we do not think it expedient to put it on the market in a solid form (except possibly as a luxury good, in a small volume). Invert sugar consumption should be based on the food industry. Although a sort of artificial honey made by the inversion and aromatization of beet sugar is marketed abroad, in Hungary even the consumption of natural honey is not satisfactory, though this may be due in part to the rather high price. Apart from the food industry, only canteens and restaurants can be expected to consume large quantities of invert sugar.

POZSÁR, B. I.: I am of the opinion thet the confectionery industry requires sugar in a liquid state, but the households will also get used to it, so the expensive crystallization would not be justified.

RUFF, J.: From the point of view of small consumers, habits have a dominant role, and the effect of fashion on the market is much lower, as the bulk of the buyers consists even today of housewives, who are very flexible as regards clothes, but all the more conservative in the art of cooking. Therefore, in my opinion, invert sugar will only be successful in retail trade in granulated form. A simultaneous change in the quality and the form of sugar would be too much and might evoke mistrust.

SHMILLIÁR, M.: In many cases households use sugar in a dissolved state. It is only a matter of habit to abandon the tiresome work of syrup preparation and use ready-made syrup instead. In my opinion crystallizing sugar at extra cost is not worth-while. In Western Europe it has been produced for some time under German licence, mainly for medical purposes. One advantage is that it is sweeter than beet-sugar, so it is required in smaller quantities. It has been welcomed in households in the United States and is used in many different ways. I think that the use of invert sugar will be introduced to some extent in Hungarian households as well. Some American experts (Flavell) expect the high fructose liquid maize syrup to be replaced later by dry sugar sold at a higher price. I agree with the opinion that a state of equilibrium will develop in the consumption of the two types of sugar.

SÓLYOM, L.: According to my present knowledge liquid invert sugar, as a trade commodity, is accepted by industrial consumers. In the households the use of granulated sugar is likely to be continued, because of the familiar way of handling and storing it.

SZÁNTÓ, S.: The crystallization of invert sugar is a much more complicated technological procedure than that of saccharose; it is more readily dissolved in water than the latter. It does not seem probable that the crystalline state will be attained in the first step.

TARJÁN, R.: The change in consumption habits is a comparatively slow process. The invert sugar syrup produced from maize will probably be used for a while as a "honey substitute", and the rate at which it begins to replace sugar will depend on how successful the advertising is. For certain purposes invert sugar sold in solution will be widely used sooner or later in the households as well. Nevertheless, the consumer demand for granulated sugar will remain high, both when the new product is introduced and later. Whether this should be produced from beet or maize is chiefly an economic question.

TYIHÁK, E.: Preference should be given to the production of solid invert sugar as it ensures better possibilities of standardization and the more efficient elimination of substances causing side-effects.

VUKOV, K.: Invert sugar is a mixture, so it cannot be crystallized.

ZELLER, GY.: I think that the majority of industrial consumers will be able to use the liquid invert sugar without much difficulty, thus the granulated form would not be economical for them; the exception will be those industrial enterprises where the use of liquid invert sugar causes technical difficulties.

The situation is different in the households. Most consumers will not be easily persuaded to change over to the use of sugar in this unusual state. Stimulation is therefore indispensable in this field.

Stimulation in this case can be attained with the price. If liquid invert sugar is appreciably cheaper and suitably packed it will be possible to raise a demand and alter the consumption habits. But this is a slow process, so I think invert sugar should definitely be produced in both physical states: in a granulated form at a much higher price, and in a liquid form at a so-called "penetration price", that is, ensuring only a very modest profit.

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PÁL, GY.: **On an industrial scale invert sugar has been produced in the United States since 1974 at an ever increasing rate; in Hungary the manufacture of invert sugar is in the phase of experimental production. Since even the nutritionists do not possess sufficient experience on mass consumption do you expect the consumption of invert sugar in a form immediately utilizable to the organism to have any useful or harmful consequences?**

ALMÁSI, E.: I think the direct consumption of liquid invert sugar will be minimal. According to the results of preliminary surveys, breweries, wineries and distilleries will be the biggest consumers of liquid invert sugar, that is, the invert sugar will reach the consumers after fermentation in the form of alcohol.

The other big consumers (canning industry, confectioneries, etc.) will use it mostly for purposes which now involve the inversion of saccharose, or where starch syrup is already used.

BAJNÓGEL, J.: I am not a nutritionist, so I cannot answer the question from a biological point of view. However, approaching the question from a consideration of consumption tendencies, I think that, reckoning with pure glucose or fructose or a mixture of the two, and supposing that sugar consumption will follow the present tendency, no harmful effect can reasonably be expected from the consumption of this type of sugar, provided that other nutrients are consumed in adequate amounts and proportions, so that deficiencies in the calory requirements of the human organism, which are undesirable from the point of view of nutrition biology, do not occur. The problem raised in the question is of great interest and therefore requires further consideration.

The consumption of liquid invert sugar has certain disadvantages, however. A certain proportion of this type of sugar may well be used in households as a syrup flavoured with various aromas for the preparation of soft drinks. Since such drinks contain no valuable nutrients apart from the sugar, their consumption is not desirable from a nutrition-biological point of view.

BENDE, P.: Liquid sugar produced from maize will be used in Hungary, as in the United States, for further processing by the food industry, but it would not do any harm to the health even if consumed directly.

BOLGÁR, P.: The components of liquid invert sugar are already consumed, so this involves nothing new and no extra load for the organism.

GALLÓ, GY.: It is true that experiences concerning the consumption of liquid sugar have only been obtained quite recently; nevertheless the usefulness of invert sugar seems to be an unambiguously proved fact.

GÁBOR, M.: The possible harmful effect exercised by invert sugar on the organism depends on various factors, for example on the additives used, the purification processes applied in the course of production, and on undesirable new compounds which may occur during the technological process (hydrolysis, heat effect). I suggest that these factors be subjected to scrutiny from the point of view of a possible harmful effect.

GÁSPÁR, L.: Saccharose is hydrolyzed in the intestine. Absorption thus takes place in the form of monosaccharides. In principle there cannot be any difference in the metabolisation of saccharose and invert sugar, as invert sugar is one stage in this process. This is especially so if the invert sugar produced contains the aldose and ketose components in an equimolar ratio, as it is produced during the inversion of saccharose.

HOLLÓ, J.: Since the human organism inverts beet sugar into glucose and fructose, the consumption of sugar obtained from maize, which is in fact a mixture of glucose and fructose, cannot involve any harmful effects, but will in fact save the organism the work of inversion.

KISFALVI, T.: Easy digestion may be an advantage, while the disadvantages are the undesirable weight increase, due precisely to this easy digestibility, and the possible presence of harmful residues from occasional Fusarium infections in the products.

LŐRINCZ, J.: Since fruit, honey and the products of the confectionery industry contain invert sugar without any harm being done to health due to their consumption, it is not probable that the effect of invert sugar produced from maize will be at all different. Nevertheless, I feel that experiments on animals before the product is introduced as a consumer good are justified.

PAIS, I.: In my opinion this question is the most important one. On the basis of experiences gained in other fields it must be assumed that smaller or larger changes in the nutrient chain may induce various disorders or even physiological diseases in people sensitive to such effects. It is therefore indispensable to carry out thorough and complex investigations before the new product is put to wider use.

PÁSZTOR, K.: Provided it does not contain any contamination I do not think that liquid sugar should exercise any adverse effect on the organism. Nevertheless, investigations will be needed to find out whether or not the herbicides, pesticides and other chemical plant protectives applied during the disease control of maize are incorporated in the grain, and whether their after-effects should be reckoned with in the course of invert sugar production.

PECZNIK, J.—MAJER, J.: From the point of view of nutrition, invert sugar can be regarded as equal in value to saccharose. Although publications have appeared concerning the harmful effect of saccharose consumption, according to our knowledge this question has not been settled so far. In fact a considerable proportion of the sugar is already consumed in the form of invert sugar: fruits and honey contain invert sugar; in factories producing soft drinks, liqueurs, preserves and confectionery beet sugar is often inverted before use, or inversion takes place during the manufacturing process or due to the acid content of the product.

POZSÁR, B. I.: The human organism inverts saccharose just as will be done by the technology of the planned factory with the addition of enzyme preparations. No harmful effects can therefore be expected. Invert sugar is sweeter than saccharose and not only its absorption but its metabolism too are more intensive.

RIGÓ, J.: The properties of invert sugar are particularly favourable in cases of great physical strain, when the organism requires easily converted and mobilized energy sources. The consumption of invert sugar is not expected to have any harmful effect, except with certain enzyme disorders which involve an intolerance to fructose, for example in the case of an innate reduction in the liver aldolase activity, but in that case all fructose-containing foods are omitted from the diet. On the other hand, invert sugar may be efficiently used in the case of intolerance to saccharose, when saccharose, or other disaccharide-decomposing enzymes are absent.

ROMÁNY, P.: The human organism utilizes beet sugar by breaking it down to glucose and fructose. Invert sugar is an approximately 1 : 1 mixture of these two sugars, so the organism is provided with what it would otherwise have to produce for itself.

SHMILLIÁR, M.: In the United States invert sugar has already been manufactured from maize for some time. Its indirect utilization and direct consumption are constantly on the increase, and experience has been gained on the correct form and rate of utilization. No references to harmful consequences are found in the publications. In the United States many bakeries, and fruit juice and soft drinks factories have switched over to using liquid invert sugar. The European countries put crystallized fructose (Casselli) on the market a long time ago. The American representative of a German manufacturing company markets crystallized fructose in the United States at 1.15—1.25 $ pound. The higher prices are compensated by the higher sweetening ability of the product, says the company's representative. An American company produces crystallized fructose in Finland (Finn-Cal Fruit Sugar Corp.). From 1976 onwards the company plans to market its products in the United States as well. Crystallized fructose is already widely available in the supermarkets, chemists and drug-stores of Europe. It is used in jam and yoghourt products, and is also available on the market in 5 pound bags. According to the manufacturers it is recommended by medical corporations, who say that crystallized fructose is much healthier than beet sugar, especially for diabetics. It seems highly probable that, considering how wide the distribution is, any harmful effects of invert sugar on the human organism would have been noticed by now.

SÓLYOM, L.: The human consumption of invert sugar is not expected to cause any adverse side-effects unless some undesirable alien material has got into the crop, which may be transferred to the invert sugar in the course of manufacturing.

SZÁNTÓ, S.: Not more than would be caused by the consumption of the saccharose which is replaced by the liquid invert sugar. Liquid invert sugar, like saccharose, is considered required for its decomposition in the organism (vitamins, mineral substances); the latter must be supplied from elsewhere.

SZÉKESSY-HERMANN, V.: In my opinion the main issue in the problems connected with the manufacture of liquid invert sugar is what the response of the human organism will be if the beet sugar (disaccharide) used so far for flavouring and preserving foods, and for preparing sweets is replaced by invert sugar (a monosaccharide mixture) or by one of its components. I should like to examine the biochemical aspect of the question from two points of view; *a)* from the point of view of the digestive tract: the characteristic features of carbohydrate digestion which may be considered physiological under the present conditions of nutrition; *b)* from the point of view of the tissues: the intermediary metabolism characteristics of glucose and fructose, the two components of invert sugar.

a) According to the opinion of the physiologists engaged in nutrition studies one of the factors of proper nutrition is to meet 50—55% of the calory requirements with carbohydrates. The bulk of this is made up by the starch content of our food. The proportion of preformed disaccharides (mainly saccharose) and monosaccharides (of fruit origin) in our food is estimated to be 10%. Nutritionists generally view the increasing consumption of refined sugars with anxiety. The idea that the per capita household consumption of sugar is a measure of the living standard has become outdated. On the contrary, a large proportion of the population in civilized countries stands in need of a protective diet, particularly to prevent the manifestation of a tendency to diabetes.

In the digestive tract the starch contained in our food is broken down to maltose (a disaccharide composed of two glucose molecules) under the influence of amylase, a product of the salivary glands and the pancreas. The sugar used for sweetening (sucrose) enters the small intestine, where absorption takes place, unchanged. Since only

monosaccharides are absorbed into the blood, in the course of absorption both the maltose that comes from the starch, and the saccharose have to be hydrolyzed into monosaccharides. However, this process does not take place in the lumen of the small intestine, but in the mucosa of the intestinal wall, where the specific disaccharides which carry out the necessary splitting are synthesized. Thus, it is clear from what has been said that under physiological conditions only a negligible amount of monosaccharide is present in the contents of the intestine. Considering the sensitiveness of the intestinal mucous membrane it is highly questionable whether the frequent consumption of liquid invert sugar would not lead sooner or later to lesions of the intestinal mucous membrane, particularly in the case of children. The transport systems of the intestinal mucosa are especially sensitive to different metabolites. It has been pointed out, for example, that the presence of fructose has an unfavourable influence on the absorption of amino acids from the intestine.

The mechanism and regulation of the speed of carbohydrate absorption have not yet been fully clarified. However, it has long been established that the monosaccharides vary both in the speed and mechanism of absorption. The intestinal mucosa is, in fact, the first barrier where the qualitative selection of substances entering the organism takes place and the rate of their absorption is controlled. The disaccharides are known to decompose in the intestinal wall to monosaccharides. Due to the compartmentalisation of the intestinal mucosa these disaccharides are found near the transport systems of their respective monosaccharides, and since their activity is also controlled by the concentration of the end-products, their hydrolyzing activity is adjusted to the functions of the transport systems. The majority of the fructose derived from sucrose is thought to be transformed into glucose in the intestinal mucosa and to reach the vena portae in this manner.

b) As to the intermediary metabolism of glucose and fructose, the earlier conceptions were based on the fact that in the human organism there are many possible ways in which monosaccharides can be transformed into one another. In the case of glucose, fructose and galactose (a component of milk sugar), for example, such transformations do in fact occur, to a minor extent in the intestinal mucosa, but more generally in the liver. Since glucose is the carbohydrate that maintains the level of the blood sugar and supplies energy primarily to the tissues, other carbohydrates only come into consideration if they are first transformed into glucose, or if through some mechanism or other they provide an intermediary product of the glucose metabolism.

The glucose level in the blood, and the glucose supply to the tissues are under complex, mainly hormonal, control, with insulin as the central link in the chain. The observation that the metabolism of fructose is independent of insulin raised the idea of using fructose in diabetes, and in certain serious conditions, mainly when a parenteral nutrient supply is required. Closer examinations have revealed, however, that the question is not as simple as that. First of all, the structural elements of some tissues, e.g. central nervous system, heart, muscles, blood, are able to utilize fructose only in very small quantities. The liver, on the other hand, takes up fructose at an even faster rate than glucose, and even forms glucose from it, through special intermediary products. One such intermediary product is fructose-1-phosphate. Although an enzyme capable of performing the further transformation of fructose-1-P is present in the liver, its activity is relatively low. If the amount of fructose in the liver is higher than the physiological level, it cannot cope sufficiently rapidly with the fructose-1-P produced, so the concentration of the latter will exceed the physiological value. The increased fructose-1-P production is not, however, without effect on the other metabolic processes in the cell. Under its influence certain adenine-nucleotide decomposing enzymes are released from the natural inhibition, thereby causing hyperuricaemia and a reduction in the ATP content in the liver cells. The latter obviously damages the liver cells.

Discussions and studies on the fructose metabolism are still in progress. A quotation concerning the per os application of fructose from the paper: Dangers of intravenous fructose (H. F. Woods and K. G. Alberti, Lancet Vol. II. of 1972, p. 1354) will serve to give some idea of this: "Similarly we feel that large amounts of oral fructose could be harmful although the quantity of fructose that can be ingested acutely is limited by its cathartic effect."

Further biochemical arguments against replacing saccharose by invert sugar or one of its components could be given. Both glucose and fructose should be regarded as substances used in therapy, or as carriers of medicines. Their regular uptake in food could lead to consequences which it would be difficult to foresee.

In my opinion the manufacture of invert sugar from maize should definitely

not reduce the extent of beet sugar production for household and food industry purposes. I think the production of invert sugar is only justified if it is not designed for direct utilization, but to supply basic material for some further product (e.g. alcohol). Of course, the production of glucose and fructose for therapy and experimentation would form an exception.

TARJÁN, R.: I have already touched upon some problems connected with the consumption of crystalloid carbohydrates. Considering that in the organism beet sugar is in any case transformed into invert sugar, from the point of view of nutrition the question is how much rather than what kind of crystalloid carbohydrate should be consumed. Thus the possible harmful consequences will be attributable not so much to the quality of the sugar as to the increased consumption due to the efficient, intensive advertising which the new — and perhaps cheaper — kind of sugar is likely to be given.

TYIHÁK, E.: Considering the prospective biological effects of raw materia lused in such large volumes, the data of the biological tests performed so far with invert sugar should be surveyed, and an up-to-date systematic biological evaluation is also imperative.

VUKOV, K.: On an industrial scale invert sugar has been produced in the United States since 1927, using saccharose as the initial material.

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PÁL, GY.: **Apart from starch, cellulose is also a polymer of glucose, and is much more widely distributed than starch, because 40—50% of the wall of young cells and 60% of that of lignified cells have been found to consist of cellulose. The total amount of cellulose in the world is estimated to be 1100 billion kg. Do you think it likely that in the distant or possibly in the near future invert sugar will be produced not from maize grains but from another sugar-supplying basic material, the maize stalk?**

ALMÁSI, E.: I do not think it likely that maize stalks will be used for invert sugar production in the near future.

BARDACH, S.: I think that the utilization of maize stalks is a very important question which must definitely be solved. However, in order to assess the possibilities of industrial utilization, the technical and economic circumstances related with the raw material supply of the future factory must also be taken into consideration.

The economic efficiency of a factory processing maize grain (compared, for example, to a beet sugar factory) is based among other things on the fact that it can be operated continuously all the year round and that the raw material can be easily transported and economically stored.

The volume of maize stalk (and of straw, wood shavings, etc.) is too high compared to its value. The costs of transportation and storage may consequently make the economic efficiency of large-scale processing doubtful. Under the present economic conditions its utilization is an agricultural rather than an industrial task, to be solved on the spot (e.g. by ensilation).

BENDE, P.: The introduction of sugars produced from other polysaccharides, e.g. from cellulose, greatly depends on the discovery of effective and economical biological catalysts.

So it is not unlikely that other polysaccharides will also be used in the future as basic materials for sugar production.

BODNÁR, M.: One of the harmful consequences of the modern technical revolution is that the richness of the organic world diminishes, its diversity becomes limited and its harmony breaks down. It is therefore of enormous importance, especially under conditions of dynamic population growth (e.g. in the third world), that all the by-products which are now more or less wasted (e.g. maize stalks) should be utilized. I think, considering the rapid progress of science and technology, that the production of invert sugar from maize stalks will be possible in the not too distant future.

BOLGÁR, P.: Sugar production from cellulose depends on the future of the cellulase enzyme, just as the isomerose syrup produced from starch results from the alpha-amylase, amyloglucosidase and glucose isomerase enzymes .

FÜREDI, J.: In my opinion further raw materials for sugar production may be found in the future. The utilization of such basic materials as maize stalks, cobs, etc. is also justified by the fact that the firm, hard stalk required for mechanical harvesting is an undesirable by-product of the present plant cultivation technology. This mass should be utilized in some way (e.g. in the cellulose, fibre board and fodder industries) in the future. The use of such basic materials in the sugar industry will, however, be greatly limited by the expensiveness of the processing techniques.

GALLÓ, GY.: I think it will be possible to produce invert sugar from maize stalks in the not too distant future, provided appropriate technological equipment is produced.

GÁSPÁR, L.: In my opinion in a maize-producing country cellulose cannot compete with starch. The planned invert sugar production has the advantage, among others, that it is a biochemical industry with a relatively low energy demand, producing sugar with few by-products that cause environmental pollution.

If sugar production is based on cellulose as raw material, not only the high energy and water demand but also the considerable effluent production involved in fibre extraction must be taken into consideration. At present all three factors are likely to exercise an unfavourable effect on the processing of cellulose-containing materials all over the world.

Research is being carried out on the use of fungal cellulases, but as far as I know no technologically adaptable results have been achieved so far. If the production of sugar from cellulose could be fully transformed into a bio-industry, then it would be worth studying the conditions of profitability.

HOLLÓ, J.: Cellulose is well-known as a potential source of human and animal nutrition, but this requires the availability, on an industrial scale, of a cheap cellulase-enzyme product, which would rapidly and economically decompose cellulose into glucose. In the future maize stalks, wood shavings or other cellulose-containing substances may serve as a potential basic material for the production of glucose-fructose syrup. Dollar calculations of the acid hydrolysis process do not give economical results and the technology is rather cumbersome. Cellulolysis may be regarded, on the other hand, as an important reserve, a potential answer to the world's sugar problem.

KISFALVI, T.: This is possible in the fairly distant future, but is hardly probable in the near future.

LÁSZTITY, R.: Cellulose undoubtedly occurs more widely and in larger quantities in the plant world than starch. At the same time, its separation from the accompanying polysaccharides and either acid or enzymatic hydrolysis represents a much greater problem than the corresponding processing of starch does. So the production of sugar from cellulose for utilization in the food industry is not likely to be the development tendency of the near future. In the distant future biochemical reactors, using nuclear or fusion energy to produce sugar from carbon dioxide and water irrespective of the weather and site conditions, may be constructed.

LŐRINCZ, J.: According to my knowledge, in the United States experiments on the use of the whole maize plant in a similar way to sugar cane are already in progress, though much still remains to be done by the breeders.

NAGYPATAKI, I.: In connection with the utilization of maize the question of processing maize stalks has often arisen. There are well developed industrial procedures by which furfural can be produced from maize stalks, and the cellulose content of the maize stalk can also be utilized in the form of glucose.

It should be noted in this connection that, on the one hand, the investment and processing costs are relatively high and on the other hand, it would not be reasonable to make large investments for maize stalk processing when there are far fewer factories for the full processing of the maize grain, which is much more valuable than the maize stalk, in the world than necessary. With a view to the rational and economical use of the resources and the acceleration of food production the available resources should be spent on establishing factories for the complex processing of the maize grain.

When the possibilities of processing maize grain are exhausted, while the demand for foodstuffs increases to a still greater extent and the technologies of maize stalk processing are further improved, then the processing of maize stalks can be initiated.

NÉMETH, S.: The extension and accumulation of scientific knowledge and experience result sooner or later in the extension of production. On the basis of results in biology, chemistry, etc. It is certain that the cellulose polymer of glucose will be used in the future as a source of sugar, and a technology for invert sugar production from maize stalks will also be elaborated.

PAIS, I.: Sugar production from maize stalks (and cellulose in general) is not impossible, but as the technology is likely to be more complicated and expensive, since cellulose is not readily hydrolyzed, it will not be of much importance.

PÁSZTOR, K.: The proper utilization of maize straw has not yet been solved. It is conceivable that the large volume of straw could be used more efficiently than by the present practice of ploughing it into the soil. It might well be suitable for invert sugar production, though harvesting and transportation would raise further management problems (harvesting and transportation capacity, etc.). However, the greatest problem in this field is to discover enzymological and biochemical processes for cellulose decomposition. Once this is solved, and it becomes possible to produce starch from cellulose, then the use of maize grain will become unnecessary.

PECZNIK, J.—MAJER, J.: Cellulose is also built up from sugar molecules (cellobiose or glucose). However, it can only be hydrolyzed with strong acids, under pressure and at a high temperature. The hydrolysis must be carried out in stages, removing the sugars produced and adding fresh acid, to prevent the sugars produced by the hydrolysis from undergoing further decomposition. It is unlikely that any method by which pure sugar suitable for human consumption could be economically produced will be found within the foreseeable future. The processing of straw and maize stalks, to make then more suitable, when enriched with NPN, for feeding ruminants, is another matter.

POZSÁR, B. I.: Besides grain crops sugar will probably also be produced from agricultural refuse with specially adapted technologies. The partial saccharification of straw, as a waste-material, for feeding purposes has already begun. Organic matter utilization from the saccharified product is improved not only by the absorption of sugars; the sugar content favourably influences the absorption of other metabolites too.

ROMÁNY, P.: It is true that cellulose is almost unrestrictedly available on the Earth and, in fact, forms a considerable part of all plants. During the last century attempts were already made to utilize cellulose in the food industry. During World War II, when there was a short renaissance of all kinds of substitutes, a sort of sugar (called xylose) was produced from sawdust by means of fuming hydrochloric acid, but the process was never undertaken on a large scale. Today nobody would think of using acidic hydrolysis, but the discovery of a cheap, cellulose-decomposing enzyme that could be used on other than a laboratory scale still remains to be made. It is probable that this enzyme will be found one day, but even then the manufacture of sugar for human nutrition from wood shavings, maize stalks and straw will not be justified, because such a large amount will never be required.

RUFF, J.: Cellulose might be used in some countries in the near future as a raw material for sugar production, since in the Scandinavian countries, for example, the refuse of wood processing plants is used for alcohol production. Whether cellulose should be processed into alcohol or invert sugar will in any case be determined by market demands.

Thus use of maize stalks as raw material will not assume large-scale proportions in the near future, in my opinion, since maize grain can be processed with higher economic efficiency.

Maize stalks and the products obtained from them by partial or complete decomposition, or by fermentation, are expected to be used primarily for feeding animals, as with the present growth rate of the human population the protein demand will substantially increase.

SHMILLIÁR, M.: It may well be possible to produce invert sugar from maize stalks, but I think that this is a problem for the distant future. I suggest that investigations be carried out on this subject, particularly to find the most economical ways of harvesting, storing, transporting and processing the stalks. The data thus obtained might be used for a comparison with the costs of invert sugar production from other materials. The proces-

sing of cellulose is an expensive solution compared to the relatively simple production of sugar from starch.

SÓLYOM, L.: With suitable industrial enzyme preparations and sugar manufacturing technologies other materials with high cellulose contents may also be expected to form the basic material for glucose and fructose production.

SZENDREY, I.: The manufacture of invert sugar from maize is, in fact, a food transformation by which the amount of one foodstuff, sugar, is increased at the expense of another foodstuff, starch. Thus in an absolute sense, the food supplies of the world population will not increase in this way. The situation is different if instead of starch the polysaccharides of the cell-wall (cellulose and the hemicelluloses) are converted into sugar. For this purpose maize stalks are suitable, among others, as are wood shavings.

Harvesting and chopping are simpler to solve in the case of maize stalks. In the case of wood the difficulties are increased by the bark, which has several disadvantages from the point of view of sugar output. The composition of the bark tissue within the same species varies greatly with the age of the tree. From the cambium outwards the ratio of saccharifiable to non-saccharifiable bark tissues gradually worsens. In addition, the colloidally dispersed outer suberized part binds the sugar molecules produced during hydrolysis on its surface, thereby decreasing the sugar output and increasing the time required to wash the sugar out.

Apart from the bark, the 25—30% lignine content in the wood also has an unfavouranle influence on the economic efficiency of wood-sugar production. The utilization of the remaining colloidal hydrolysis lignine has not been reliably solved as yet, in spite of the large number of patents.

When processing maize stalks only the lignine problem has to be reckoned with, but as it is present here in a lower percentage than in wood, the conditions of extraction are somewhat more favourable.

The relatively high (25—30%) hemicellulose fraction causes trouble in the case of both maize stalk and wood. It is responsible for the less uniform composition of the sugar hydrolysate compared to that obtained from maize grain. Besides glucose large quantities of other hexoses and even of pentoses are produced. Among the hydrolysis products from pine species less pentose is found, while in the case of deciduous trees and maize stalks it is produced in considerable amounts.

Apart from this, the hemicelluloses are more readily hydrolysed than cellulose. Consequently, the production technology is more complicated than that of invert sugar production from maize.

In Hungary attempts were made to industrially utilize the high pentosane content of the corn-cob. The aim was to obtain furfural, a valuable decomposition product of the pentose sugars produced. At that time it was impossible to imagine a development in agricultural technology which would eliminate the corn-cob as a waste-product of combine harvesting. When planning the new furfural factory now under construction it therefore seemed more advisable to reckon with the deciduous trees growing in Hungary as basic material. With maize stalks the changes which have taken place with respect to the corn-cob would have to be taken into account.

TARJÁN, R.: This could well happen. The transformation of cellulose into glucose is theoretically possible and virtually so technologically, and this method would open up wide vistas for the satisfaction of the world's food requirements. It is another question whether the sugar obtained should be made directly suitable for human consumption, or whether it would be better to transform it biologically and gain compounds more valuable from a nutritional point of view, e.g. protein, by cultivating yeast on the sugar obtained during hydrolysis.

TYIHÁK, E.: This is not to be expected.

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PÁL, GY.: **In glucose or the invert sugar solution produced in the course of acid hydrolysis on starch and beet sugar a so-far unknown material appears which considerably shortens the life of bees; bees fed on the invert sugar thus produced are killed in 4—6 days in spite of the fact that the chemists state that the complete hydrolysis of starch with acids of low concentration produces exclusively glucose. Do you not think that during the production of invert sugar from maize starch by fermentation in an acidic medium so-far unknown materials harmful to the human organism may also be obtained?**

ALMÁSI, E.: Invert sugar can be produced from starch in various degrees of purity. With up-to-date techniques it could be made completely free from accessory substances.

BAJNÓGEL, J.: The experience obtained in the course of experiments carried out with bees fed on invert sugar is very noteworthy. The clarification of this problem would greatly help in giving an answer to the previous questions.

During the hydrolysis of starch certain toxic substances as yet unknown may be produced, or a small quantity of free acid may be left behind in the solution. This is a factual question which only chemists are competent to answer.

It may well be asked whether the bees died of some unknown toxic material contained in the invert sugar solution, or due to the lack of something which is present in honey. If the latter holds true, then the former supposition may not be correct. If, however, the first supposition is correct, then the crystallization of invert sugar, its purification and refining through further technological procedures, are even more justified.

BARDACH, S.: I cannot approach the question from the point of view of chemistry and biology. In forming an opinion I can only fall back on the fact that in the United States of America liquid sugar has been manufactured from maize since 1969. The present level of production is half a million tons a year, which is expected to exceed two million tons in the near future. On the model of the United States invert sugar factories are being constructed in other developed capitalist countries as well. I have definite knowledge of the fact that two factories in England, with a total capacity of some 400,000 ton/year, will be starting to produce invert sugar this year.

BENDE, P.: No substance with an adverse biological effect on bees is known to be produced during the hydrolysis of starch. In my opinion the example referred to raises problems connected with the artificial feeding of bees and with the contamination of artificial nutrients, and cannot be attributed to any product of starch hydrolysis or saccharose inversion.

There are no substances harmful to the human organism in the liquid sugar produced by processing maize.

BOLGÁR, P.: The adverse effect exerted on bees cannot be characteristic of man.

FAZEKAS, S.: Of the two basic materials for sugar manufacturing mentioned in the question, sugar-beet has an advantage over starch as basic material from a technological point of view. Sugar-beet cells contain saccharose in a dissolved state, and in the juice obtained from the slices only 10—15% non-sugar accompanying materials (salts, pectin, proteins, etc.) are found. In maize the starch (amylum maidis) is organized into particles, and its basic material, glucose, occurs in polymer form. The starch grains contain 0.5—2% nitrogenous material, 0.05—0.5% ash component (including phosphate and silicic acid) and 10—15% water.

When comparing the technologies for processing disaccharide beet sugar and starch monosaccharides it may be said that from a chemical point of view the present method of beet sugar production is a mild, elegant and unobjectionable procedure, which consists only of physico-chemical treatments: the concentration, decolourization and crystallization of the sugar after the above-mentioned contaminations have been removed, and in no case needs intervention causing a change in the chemical structure, or the occurrence of secondary reactions. The scalding, distillation and steaming of beet slices involved in the process may be considered as sterilizing enzyme inactivation processes which exclude the possibility of fermentational side-processes.

The decomposition of starch on an industrial scale is carried out by cheap, low concentration acid hydrolysis (more often with the cheaper, oxidizing H_2SO_4, which causes more problems, than with HCl, which induces fewer secondary reactions). Yet, under laboratory conditions the formation of by-products can be demonstrated even with 1 N HCl. The formation of by-products can be demonstrated using the hydrolysis of the commercially available, refined "Mokka" cube sugar. The experimental sample contained 0.21—1% non-sugar substances. It is worth mentioning that in saccharose the glucose-fructose glycosidic bond is hydrolyzed 500-times faster than that of trechalose disaccharide.

The figure shows that the hydrolysis of saccharose can be followed in UV light, and that its spectrum increases with time, disappearing completely after about 40 hours.

The figure shows the hydrolysis of a saturated saccharose solution in 1 N HCl at room temperature, immediately after solution, 16 hours later, and after 40 hours when the hydrolysis is completed. In a 100°C water-bath the HCl hydrolysis of saccharose solution results, within 5 minutes, in the formation of by-products of an intensive yellow colour.

In the hydrolysate, although it is colourless at room temperature, the formation of by-products can be demonstrated by gel filtration. Parallel to the period of hydrolysis, 0.5—2.0% heterogeneous material can be separated, with a molecular weight larger than that of the monomer or dimer sugars.

The hydrolysis of starch grains is likely to lead to the formation of still more by-products. Considering that starch contains many more accompanying materials it is desirable to analyse each phase of the reaction in a similar manner, and to follow the biological effects of the by-products on bees according to the test in question.

It would also be desirable to examine the accompanying materials present in maize starch after non-hydrolytic separation. In producing invert sugar, or glucose, from maize starch the coarse grist obtained from maize is used as starting material rather than starch grains, in order to reduce the surplus costs. The hygienic control of each phase of production is therefore increasingly important, and the "bee-test" seems to be biologically suitable for this purpose.

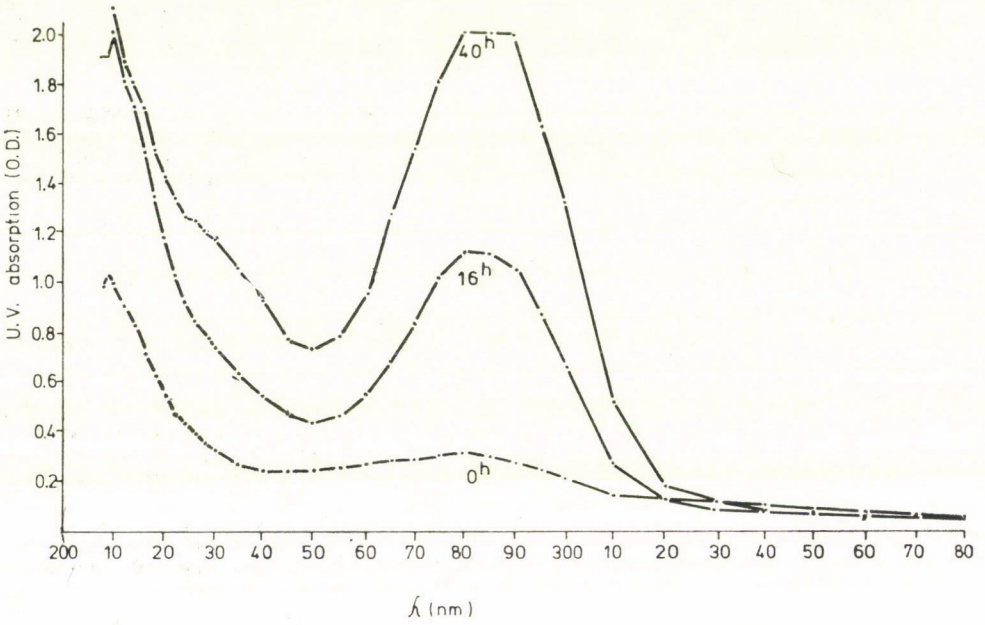

Fig. 1. Changes in the hydrolysis of saccharose, based on checking the UV-spectra at different times.

GALLÓ, GY.: I do not think it likely that substances harmful to the health are produced in the course of acid hydrolysis.

GÁSPÁR, L.: I am not familiar with the question of poisonous substances arising during the production process but it seems to me improbable; it is more likely to have been some kind of contamination at a stage when some chemical agent was used. The enzymological process itself is an in vitro form of the process taking place in nature in living organisms, and provides no grounds for suspecting the presence of harmful compounds here. The question must, however, be examined with the greatest possible care as it is a cardinal point of the whole problem.

HOLLÓ, J.: Using the present technology, starch is not hydrolyzed by acid hydrolysis. as in the case mentioned, where the bees were probably killed by reversion products formed in the course of acid hydrolysis. According to our present knowledge, no harmful by-products are formed in the course of the new technology. The optimum pH value for the isomerase enzyme which converts glucose into fructose is 8.5, but the process is run at a pH of 7.5—8, which means that no acid medium is involved. Up to the formation of glucose the technology is exactly the same as the procedure used for glucose production in the pharmaceutical industry.

HORVÁTH, GY.: I myself am a chemist, but when using either maize or starch as the starting material for fermentation experiments I found that the wash acidified from pH 5.0 to pH 2.0—2.5, with the result that most of the yeast cells died and the fermentation stopped. Fermentation was only continuous when the acid produced was successively neutralized. The addition of nitrogen salts increased the acidification, while the addition of urea had a favourable effect, presumably due to the $HI^{(+)}$-uptake by free amino groups.

This process causing acidification will not be favourable for the human organism either.

KISFALVI, T.: Theoretically no, but in practice yes, owing to contaminations, such as the remains of Fusarium infections, for example.

LÁSZTITY, R.: The acid hydrolysis of starch, and of poly- and oligosaccharides in general, especially at high temperatures, always involves the danger of secondary reactions (e.g. the formation of hydroxy-methyl-furfural, reversion products, etc.). Hydrolysis or isomerization performed with pure enzyme preparations ensures a controlled chemical transformation under the mildest conditions, so from this point of view it definitely creates a more favourable situation than chemical procedures.

LŐRINCZ, J.: The food law now in effect protects the consumer's health; for this reason I think it necessary to analyse and apply the results of experiments set up after thorough preparatory work. I suggest starting experiments to this effect as soon as possible.

NAGYPATAKI, I.: Reports have been published on the adverse effect of liquid sugar on bees. It must be remembered in this connection that bees were not designed to consume a mixture of chemically pure dextrose and fructose.

Nectar, a sugar-containing product exuded by flowers and other plant parts, is more or less an aqueous solution of saccharose, which is enzymatically decomposed into dextrose and fructose in the digestive tract of bees; in other words, it is inverted. In these sweet juices formic, malic and citric acids are usually found, and these initiate the inversion process.

The fresh honey collected by bees is nothing more than a saturated sugar solution usually containing 78—82% dry matter. Though the composition of the sugar contained in fresh honey varies within fairly wide limits depending on the plant or flower the honey was collected from, in general it contains 22—24% dextrose, 32—49% fructose and a maximum of 10% untransformed saccharose.

Besides sugar and water honey also contains some 1—3% complementary substances including pollen residues, other residues of plant origin, organic acids, proteins, amino acids, enzymes and a certain amount of minerals, which are the carriers of definite biological effects.

These natural matters found in honey are, quite understandably, missing from the liquid sugar produced from maize. It is therefore highly probable that their absence causes the unfavourable effect on bees.

Apart from this the organism of the bee produces the invertase enzyme which carries out the inversion of the saccharose in the nectar collected by the bees. When pure dextrose or fructose, or a mixture of these are introduced into the organism of the bee this enzyme-producing and saccharose-inverting mechanism cannot function; that is, if liquid sugar is consumed a very important physiological function of the bee is inhibited.

NÉMETH, S.: It is possible that when invert sugar is produced from maize by fermentation in an acidic medium other, as yet unknown substances harmful to the human organism

also arise. However, considering the rapid progress of science and technology today, their elimination can be solved within a short time.

ÖRÖSI PÁL, Z.: The substance formed during acid hydrolysis which is harmful to bees is "hydroxy-methyl-furfural", known as HMF. Any food containing more than 3 mg/100 g of HMF shortens the lives of bees. The injury occurs mainly in periods when the bees cannot fly, and are thus unable to empty the rectum.

According to the results of the experiments carried out so far the invert sugar produced enzymatically from starch is suitable for the nutrition of bees. To supplement the food of bees two sugar mixtures made from maize enzymatically have recently been introduced abroad. They are:

1. Isomerose "r", produced in the United States of America under Japanese licence. It contains 21% water and 79% dissolved matter. Of the latter 50% is glucose and 42% fructose. The total amount of other sugars makes up 8%.

2. "Apirève" (bee's dream), a mixture of maltose and glucose produced in France, following experiments at the "Institut de la Recherche Agronomique". Since last year the latter has also been advertised in apicultural journals in the German Federal Republic. It is also available with protein and vitamin supplements.

These sugar mixtures are suitable not only for the complementation of the bees' food, but also to provide more honey for sale, since apart from the honey surplus apiculturists may also remove the bees' own food requirements from the hive, and then replace or complement this with cheaper sugar. This is justified partly by the deterioration of bee forages, and partly by the damage caused by chemical plant protection to this branch of the economy.

It may happen, however, that some of the sugar fed to the bees becomes mixed with the honey. This may well occur in the hive without the apiculturist trying to "adulterate" the honey. For example, if the brood increases some of the food in the "brood chamber" may be transferred by the bees to the "honey chamber".

Sugars which resemble honey may give rise to abuses, or mislead the buyer. For example, in delicatessens in the United States of America Isomerose "r" was sold under the name "Ho-Nee". Such possibilities could be reduced by marketing the new sugar in a solid state rather than in a dissolved, bottled from for households.

In the United States the honey chemist Jonathan W. White has still not succeeded, after 2 years, in elaborating an unobjectionable chemical procedure by which all types of honey could be distinguished with absolute certainty from invert sugar. This can be explained by the fact that the chemical composition of natural flower honeys varies a great deal depending on the origin.

The question of to what extent sugar produced enzymetically from starch will be used in feeding bees depends, among others things, on whether it will be available at a reasonable price.

PAIS, I.: I do not think that the composition of the end-product in a technology of this sort can be defined unambiguously on the basis of theoretical considerations alone. The observations referred to concerning bees warn us that thorough and complex investigations should be carried out.

PÁSZTOR, K.: May it not be that the unknown substance is related with various chemical substances applied in the course of cultivation technology and plant protection, which have been incorporated into the grain?

PECZNIK, J.—MAJER, J.: We are of the opinion that the complex nutrition of man cannot be compared with the highly specialized nutrition of bees. It is probably not the invert sugar itself, of the presence of substances always found in natural honey apart from invert sugar (proteins, amino acids, enzymes, minerals, pollen, etc.) that causes the bees to die. If the consumption fo invert sugar is still felt to be risky we suggest that relevant experiments be carried out on mammals (e.g. rats).

This would be advisable in any case because, as is known from the literature, in the course of the industrial decomposition of starch by acid or enzymes a 100% glucose production is never attained, owing to the secondary reactions. The maximum DE (dextrose equivalent) value attainable with acid decomposition is 88—92%. In the secondary reactions the decomposition of glucose (producing hydroxy-methyl-furfural, and from this levulic acid, formic acid, difuryl-ether and various dyes) and the formation of so-called "reversion products" (isomaltose, gentiobiose, cellobiose, nigerose,

trechalose, laminaribiose, koyibiose, etc.) must also be reckoned with. In the case of enzyme hydrolysis, especially when saccharification is carried out with amyloglycosidase of low transglycosidase content, the DE value is much more favourable (95—98%), which naturally means a reduction in the by-products as well.

PÉNZES, L.: In the course of hydrolysing starch and beet sugar (saccharose) in acidic media a substance is produced which, according to the observations, considerably shortens the life-span of bees. If this statement is reliably confirmed on several occasions, the technology in question cannot be regarded as acceptable.

Nectar, which is more or less an aqueous solution of saccharose, is the most natural element in the life of bees. This saccharose solution is "inverted" in the organism of the bee. Yet, the industrial inversion carried out with acids has, according to the relevant publications, an adverse effect on bees. The contradiction can only be explained by the possible formation of substances during the industrial procedure which act as cross-linkers in the premature destruction of bees.

The cross-linkers play an extremely important role in mortality. Today it is an established fact that there is a close correlation between the accidental occurrence and multiplication of cross-linkers and the life-span. It is also known that the cross-linkers in question may cause not only some kind of damage to the DNA molecules (through so-called two-function damage or helix distortion), but also irreversible changes in any part of the organism. These sooner or later lead to distortions in the basic functions of the cells, then to the destruction of the cell or organism itself.

If an attempt is made to answer the question of what these substances are (which may give reactions both with nucleic acids and with various proteins, or more precisely with their reactive groups), it must be said that both the intermediary metabolites produced in the organism and substances which enter the organism from without may act as such agents. So the different aldehydes, the products of fat oxidation, certain alkylating substances, quinones, free radicals, polybasic organic acids (for instance citric acid, which is readily produced from fructose by bacterial means), etc. may all threaten the normal course of cellular metabolism in the form of efficient cross-linkers.

To sum up: I suggest the correctness of the statement formulated in the question be checked by follow-up examinations, after which, in possession of observations made on species genetically close to man, a correct decision may be made.

POZSÁR, B. I.: In the case of acid hydrolysis traces of acid may be left behind in the product (e.g. starch sugar) and these might cause the described damage; in the human organism they are found to cause damage to the teeth. In bees these same accessory substances may be responsible for the observed toxic effect.

ROMÁNY, P.: The death of bees kept on invert sugar for 4—6 days may have been caused by trace elements, e.g. lead, in the acid used for hydrolysis. It is also known that with acid hydrolysis the process does not go far enough to result in 100% glucose; besides the glucose produced, intermediates are left behind, which in an acidic medium produce various decomposition products, some of which may have a toxic effect on bees. It is also probable that the bees were killed not so much by the presence, as by the absence of something in the sugar: e.g. vitamins, enzymes, mineral salts.

According to our present knowledge, no by-products hazardous to human health can occur in the production of invert sugar using the enzyme technology. Insect organisms cannot be compared to the human organism.

RUFF, J.: In the glucose or invert sugar produced from maize starch by the acid-enzyme, or the enzyme-enzyme procedure, substances harmful to the human organism cannot, in my opinion, arise, as these procedures are in part models of processes taking place in nature.

If we only consider the human organism the decomposition of polysaccharides is started in the digestive tract by low pH gastric juices and completed by the enzymes of the gastric juices at such a level as to make them utilizable for the organism. It is true that industry uses enzymes of bacterial or fungal origin, but no toxic by-effects are known from the literature to be exercised by them on the human organism.

In my opinion the reason for the destruction of bees by industrially produced glucose or invert sugar is to be found in their digestive organs. In Hungary glucose has been produced for nine years on an industrial scale with an acid-enzyme technique, and no harmful effect has been observed in the course of human consumption; on the contrary, in many cases its consumption is medically recommended.

SÓLYOM, L.: The production of glucose from starch by enzyme hydrolysis is a technique known for several decades all over the world. No similar experience has as yet been gained concerning the isomerization of glucose. According to our present knowledge with careful isomerization techniques substances harmful to the human organism are not produced.

SZÁNTÓ, S.: The bees probably died from the absence of minerals and vitamins rather than from some unknown component.

SZENDREY, I.: If the invert sugar solution is left to stand the reversion of molecules may occur, in the course of which not only dimers, but trimers, tetramers, etc. may also be produced. Bees are unable to break down these oligosaccharides, and sooner or later they die from the invert sugar.

For the human organism these large-molecule sugar fractions do not hold any danger. It is just possible that in the course of fermentative transformation taking place in an acidic medium compounds more or less harmful to the human organism could also arise, since maize starch is not a chemically pure substance. In the course of fermentation the accompanying substances, which may include biologically harmful compounds, also undergo structural changes. Some furfural might also be produced, not only from pentoses but also from polyuronides. The starch content of maize should be increased at the expense of its protein content, as a high protein content is favourable to fungal diseases. During the hydrolysis of such infected maize grains cancerogenic substances may be formed by certain fungus species.

TARJÁN, R.: The fact that in the course of enzymatic decomposition substances as yet unknown may be produced should be given great attention and should be the subject of intensive research work. From the fact that bees fed on this sugar die in 4—6 days it is very difficult to draw conclusions pertaining to higher animals or humans. Once the problem becomes more topical I would suggest the setting up of experiments on warm-blooded vertebrates parallel to the food technological investigations in order to clear up the problem.

TYIHÁK, E.: In all probability if starch is completely hydrolyzed glucose is the only product, though this is so only when absolutely pure starch is used. The starch used so far during the examinations may not have been pure, but may well have contained small quantities of accessory materials from which toxic substances might have been produced during acid hydrolysis. This is one of the greatest problems of invert sugar production, which must definitely be clarified, due to the possibility of future mass consumption. Extensive chemical, biochemical and toxicological examinations may produce an answer concerning the nature of the toxic substance. The production of invert sugar in a crystallized form (though more expensive) may render it possible to eliminate the harmful accessory substances. Bees are well suited for use in the toxicological examinations for the isolation of toxic substances.

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PÁL, GY.: **In contrast to the high labour intensity of sugar-beet production, the shortage of sugar on the world market and the increasing price of sugar, the maize grain crop is available at a comparatively low price and in abundance for the invert sugar industry, and the extent of production can be adjusted to the sugar demand. Considering the demand for invert sugar and the fact that this is a new manufacturing procedure which requires new technical equipment, at what percentage of the beet sugar price would you fix the price of liquid invert sugar with a 70% dry matter content?**

BAJNÓGEL, J.: In the absence of data on the production costs it is difficult to make suggestions for the retail price of liquid invert sugar. I think in the present case the cost of the new production technique and of the establishment of the factory cannot be a decisive factor; the price should be determined rather by a comparison with beet sugar and on the basis of the established price structure in Hungary. This latter is particularly important because the product must be made acceptable on the market. The price is an essential factor in introducing a new product on the market. On these grounds, and considering the sugar content and sweetness of the solution, I would fix the retail price of liquid invert sugar at 60—66% of the retail price for beet sugar.

BARDACH, S.: On the basis of trial production in Hungary and of information obtained from abroad the sweetening value of the liquid sugar which will be manufactured at Szabadegyháza is proportional to the dry matter content of the product (70%).

The costs of adjusting to the liquid state are expected to be compensated by the advantageous composition of the new product, and by the omission of redissolving. On this basis I think it reasonable to fix the price of the new product at 70% of the beet sugar price.

At a wholesale price calculated on this basis, manufacturing would be economical.

BENDE, P.: The quality of the liquid sugar obtained from maize does not justify a price different from that of beet sugar.

However, with a view to stimulating industrial utilization it would be reasonable to fix a somewhat lower price for liquid sugar (calculated as dry matter content), for example 90—95% of the current price of beet sugar.

BODNÁR, M.: Under socialist conditions the prices fixed for individual products are determined by the joint effect of a number of factors: the total quantity and structure of manual and mechanical work input, national economic interests, human (social) judgement of the market value, the utilization value of the product, etc. This is naturally the case with invert sugar as well.

The wider range of application of invert sugar and its greater importance in healthy nutrition would justify fixing the price above that of beet sugar, while the lower cost of production (manual and mechanical work input) and social preference, which may be taken into account, would suggest a price much lower than that of beet sugar. Considering, however, that the new technical equipment involves an increase in costs, I think that especially in the initial period the price of invert sugar should be fixed at the same level as beet sugar, and later, with a view to developing healthier nutrition habits, lowered to 80—85% of the current price for beet sugar.

I should like to add that even if the price of invert sugar is identical to that of beet sugar this is still advantageous to the national economy, as the much lower production costs mean that invert sugar manufacture will require little or no state subvention.

BOLGÁR, P.: I agree with the standpoint of industry that the liquid sugar produced from maize should be calculated in dry matter and its price fixed at 90% of that paid for refined granulated sugar.

FÜREDI, J.: A many-sided, concrete economic analysis, a correlation analysis and a knowledge of development trends would be needed in order to answer this question.

It must not be forgotten that in Hungary maize is a very important mass fodder for the production of meat, a commodity much sought after on the world market. Therefore it is not at all likely that the maize grain crop will be unrestrictedly available at a low price in the future. This double demand and the increased costs of large-scale production might well increase the price of maize grain.

At the same time, the factors shaping the present wholesale price of beet sugar, and the development trends of these factors should also be known. In the absence of such information the price ratio of the two kinds of sugar cannot be realistically determined, though some guidelines may be given by the present and future interests of the national economy, and by the world market demands for the two kinds of sugar.

GALLÓ, GY.: I suggest fixing the price of an invert sugar product containing 70% dry matter at 60% of the price of sugar currently produced from sugar-beet.

GÁSPÁR, L.: I do not wish to discuss the price problem in detail; it cannot be decided by estimation but requires careful cost calculations. Nevertheless, I should like to emphasize two points:

The profitability calculations cannot be based on a comparison between the sugar output (about 52 q) obtained from 36 t/ha sugar-beet with a 15% sugar content and the 24 q invert sugar obtained from a 4 ton/ha maize crop, because, as has been repeatedly mentioned, during the production of sugar from maize a number of by-products equal to or more valuable than the main product are obtained.

Finally, I should like to emphasize that it would not be wise to make this industry dependent on the import of emzymes necessary for the enzyme chemical processes. Therefore, in the interests of reliability, profitability and development possibilities it would be expedient to organize the production of enzymes on an industrial scale.

GYŐRVÁRI, I.: As I have already mentioned, in my opinion we cannot speak of a permanent sugar shortage on the world market. I should approach the problem from a different side. The price fluctuations on the world market certainly justify the tendency to self-sufficiency, since the price losses caused by speculation in a single year may amount to 100 million even with imports of only 100 thousand tons. On the other hand, knowing the problems of sugar-beet production and the sugar industry in Hungary, I think that imports are unavoidable if the sugar consumption is to be increased at the same rate as in the previous decade. Besides ensuring a reliable domestic supply, the manufacture of invert sugar would offer other possibilities too. Since production can be controlled by the degree to which the capacity is exploited, in favourable crop years larger volumes of beet sugar could be made available for export by the proper adjustment of the retail price ratios (for invert and beet sugar). Concrete suggestions cannot be made for the adjustment of the price ratios, as this depends on the current conditions on the world market. In a balanced world market situation I think that a 40—50% lower price compared to that of granulated sugar would stimulate the household consumption of liquid invert sugar. However, it is the difference in the production costs between the two kinds of sugar that should be decisive.

HOLLÓ, J.: I agree that sugar-beet production is very labour-intensive, but I am not convinced that there is really a shortage of sugar on the world market. Naturally, if we consider the starving populations of underdeveloped countries as potential sugar consumers, there is a sugar shortage in the world, but as regards the sugar consumption of affluent societies, there seems to be a surplus rather than a shortage of sugar in the world, according to this year's statistics. This is also evident from the gradually decreasing trend in sugar prices observed during the past two years. In spite of this, the production of sugar from maize is an economic proposition. Under domestic conditions, the production costs are lower than for beet sugar. The price of beet sugar can only be maintained at the present level by means of subventions, while, according to the plans, the production of sugar maize will involve a certain amount of profit. As against the present price of 15 Ft/kg for subventioned beet sugar, I would consider approx. 9.40 Ft to be a reasonable price (without subvention) for glucose-fructose syrup with a 70% dry matter content.

HORVÁTH, GY.: I would suggest fixing the price for liquid invert sugar with 70% dry matter content at 10—11 Ft/kg.

KISFALVI, T.: In several respects the question is not sufficiently precise, not quite accurate. It makes quite a difference whether we only consider the domestic demand for invert sugar, or reckon with the foreign market demand too. It is obvious that the inland price of invert sugar is meant here, though this is not expressed in the question. It is not clear, on the other hand, whether a proposal is to be made for the producer's, wholesale or retail price, although there is no reason why these three prices should be fixed according to the same principles. These circumstances make it difficult to give an answer.

Owing to the export interests of the national economy foreign market demands cannot be left out of consideration when determining the inland prices. On the world market the price of liquid invert sugar is generally 15% lower than that of beet sugar, calculated on a dry matter basis. In establishing the domestic producer's price I suggest that this lower world market price level should be left out of consideration, with the view of encouraging exports, since it is well-known that in Hungary the maize industry has not yet been established, so development should be encouraged.

The wholesale price could follow the producer's price, that is, it could be identical to the wholesale price of beet sugar. The retail price, on the other hand, ought to be 5—10% lower, at least during the first 5—10 years, than that of beet sugar with a similar dry matter content. It is another question what price policy should be followed after the successful introduction of the product; this is a long-range subject, however, and many new aspects may arise in the meantime; the domestic and foreign market conditions may also change to a considerable extent. The above-mentioned savings in transport costs and energy consumption will undoubtedly compensate the national economy for the initial lower retail prices.

LŐRINCZ, J.: It is erroneous to assume that maize is available in unlimited quantities. In Hungary maize is primarily used for feeding animals. The maize supply ought to increase

parallel with the increase in the number of livestock. Only the volume in excess of this can be reckoned with as a raw material for sugar production. In my opinion the necessary balance could be maintained with an invert sugar price equal to that of beet sugar.

Since the relevant calculations are to far based only on hypotheses, it would be advisable to wait for the results of large-scale production before fixing the price.

As a matter of interest it may be noted here that with our present price system the gross returns from sales of a 350—400 q/ha sugar-beet crop is 30—32,000 Ft, while that of a 70—80 q/ha maize crop is 20—24,000 Ft. This calculation is naturally only approximate, so a detailed economic analysis of this question is certainly necessary.

I should like to add that from 1 q maize grain, which costs 280—300 Ft, 37,5 kg invert sugar can be produced by dry fractionation, while the same amount of sugar can be obtained from 3 q sugar-beet, which costs only 240 Ft (80 Ft/q). Complex economic calculations should therefore be made concerning the dry and wet procedures of invert sugar manufacturing.

Finally, I think that the value of the by-products left behind when using sugar-beet and maize as raw materials, and up-to-date methods of utilizing them ought to be examined as well. It would also be worth considering whether cheaper import sugar could not be obtained by exporting any possible surplus yield of maize.

NAGYPATAKI, I.: When determining the price of liquid sugar compared to that of beet sugar both the production costs and the useful properties, i.e. the utility market demand and market value, must be taken into consideration.

An objective judgement can only be based on a knowledge of the origin, production conditions, quality and biological value of the new product, and on the possibilities of utilization in comparison with the characteristic features of other already known and used sugars (e.g. beet sugar, honey).

When making a comparison it must be taken into consideration that these sugars are of natural origin and can be extracted or produced in a pure powdery, crystalline or liquid state from raw materials of plant and animal origin.

There are many raw materials of natural origin (fruit, vegetables, grapes, milk, honey) from which sugar could be extracted but these quite reasonably are consumed directly as foodstuffs.

Other natural products e.g. cereals, including maize, potatoes, manioca, ect. are consumed directly, using various means of preparation, but these can also be subjected to industrial processing, and pure concentrated sugar products can be obtained from them.

There is a third group of natural raw materials which cannot be directly consumed as food (sugar-beet, sugar cane), but which can be industrially processed to produce sugars suitable for human consumption.

It should be noted that grains and tubers containing starch (maize, cereals, rice, potato, manioca), fruit containing simple sugars mainly in the form of dextrose and fructose, and honey were the original carbohydrate nutrient sources of the human organism rather than saccharose obtained from sugar cane and sugar-beet. Man only began to extract saccharose (beet sugar, cane sugar) from plants much later, largely under the pressure of necessity.

Dextrose and fructose, the components of liquid sugar, occur in nature in an extraordinarily rich variation, either separately, or in the form of large molecule polymerization derivatives (starch, cellulose, inulin, glucogen), or even as natural mixtures of the two (fruit, honey).

In comparison beet sugar (saccharose) makes a very poor showing in the plant world, as regards both quantity and diversity of forms.

No polysaccharide is known in nature which yields beet sugar (saccharose) by decomposition. In human and animal organisms beet sugar (saccharose) does not occur at all; when introduced with nutrients into the digestive tract it is immediately decomposed in the course of intensive enzyme activity. The blood circulation which supplies the living organism with nutrients does not transport saccharose at all. Under the natural conditions prevailing in the living world (in the presence of water, organic acids, enzymes and gastric juices) saccharose is unstable and either decomposes, or is present as the equilibrium product of two-way reactions.

This all goes to show that in the living world saccharose (beet sugar, cane sugar) is a compound of peripheral importance in the carbohydrate group.

On the other hand, dextrose and fructose, the components of liquid sugar, are compounds of central importance, indispensable for living organisms in general, and for

the human organism in particular; they are transported by the blood system, take part directly in physiological processes and can be transformed into one another, but they cannot be replaced by anything else.

Thus, owing to the high diversity of its occurrence in nature, and due to its central role in the living world and particularly in the human organism, the mixture of dextrose and fructose marketed as liquid sugar is of higher biological value than saccharose (beet and cane sugar).

Since the sugars are mainly used as sweetening nutrients for human consumption, in judging their utility their biological value is of the greatest importance.

For this reason, if beet sugar has been able to spread to such an extent despite its peripheral biological role, nothing can prevent liquid sugar (a mixture of dextrose and fructose) from gaining markets all over the world.

When comparing beet sugar with the liquid sugar obtained from starch, attention must naturally be paid to the important fact that in contrast to the limited properties and forms of beet sugar a highly diversified range of sugars can be obtained from maize as regards their chemical composition, physical state, outer appearance and other properties, the utility and production costs of which vary considerably.

From the rich selection of sugars obtained through the decomposition of maize starch mention may be made of the syrups produced for the confectionery industry powdery maltirons (maltodextrine), malt sugar, powdery dextrose and its crystalline form: granulated dextrose, bulk dextrose, and liquid sugar, a mixture of dextrose and fructose wrongly called invert sugar (it is not produced by inversion), the subject of our investigations.

Moreover, liquid sugar can be produced with different composition ratios according to the demands of the consumer. For several years a form of liquid sugar containing 50% glucose, 42% fructose and 8% compound sugar has been marketed. Today this is the kind of liquid sugar sold in the largest quantities.

Liquid sugar in which the fructose content has been increased to 55%, the glucose reduced and the proportion of compound sugars lowered to 3% has recently has put on the market.

There is also a method of liquid sugar production whereby the fructose content is increased to 90%: that is, in this glucose-fructose mixture fructose is the determinative component.

Since glucose and fructose have different solubilities, crystallization and sweetness, the most diversified liquid sugars can be produced by changing their ratio to one another.

The specific features of the glucose and fructose found in liquid sugar are in fact complementary to each other and give favourable results in different foods: confectionery products, jams, conserves, beverages, icecreams, etc. They ensure the sweetness, flavour and hygroscopy of food products, keept the freezing point low and the osmotic pressure high, make food products fermentable and alcohol-containing beverages soluble, and promote browing, which is an important processing factor in confectionery products.

Since sweetness is a characteristic feature of sugar, it is important to know that taking the degree of sweetness for 100 in the case of saccharose (beet sugar or cane sugar), it is 70 for glucose and 130 for fructose. Therefore the degree of sweetness is also 100 in liquid sugar containing glucose and fructose in nearly equal proportions.

On the basis of the above it can be seen that the useful properties of liquid sugar are much more numerous and important than those of beet sugar, so liquid sugar is more valuable to the user and could thus be made more expensive than beet sugar. On the other hand, the large volumes of maize raw material available render it possible to produce liquid sugar at lower costs, provided factories with optimum capacities and up-to-date equipment are built, and provided the maize is complexly processed to give a well considered range of products.

The advantage to the national economy consists in the fact that liquid sugar of higher utility is cheaper to produce, while in factories carrying out further processing it can be processed at a lower cost than either beet or cane sugar.

If the full starch content of maize is used for sugar production, 60 kg pure sugar can be obtained from 100 kg shelled maize.

On the basis of the utility and production cost of liquid sugar it would be reasonable to put it on the market at the price level of beet sugar.

However, considering that a new product is to be introduced, it is worth stimulating its distribution with a favourable price.

I suggest fixing its price at 90% of the price of beet sugar. A price higher than that would probably check its rate of distribution, while a lower price would limit the possibilities for fund accumulation by the producers and thereby the rate at which further maize processing factories will be established.

NÉMETH, S.: If sugar production from maize is to be made competitive, the price of liquid invert sugar with 70% dry matter content should be fixed at 55—60% of the price for beet sugar.

PÁSZTOR, K.: The price is influenced by the maize production costs, the manufacturing technology, the amount of invert sugar extracted and other factors. Only with a knowledge of these factors could the price of invert sugar be determined.

PECZNIK, J.—MAJER, J.: To a first approximation it seems reasonable to fix the price of invert sugar, calculated in terms of pure sugar, at the same level as that of beet sugar. A higher price would have an unfavourable effect on the demand, while a lower price would suggest poorer quality. With a price identical to the price of beet sugar the advantages listed above would ensure a satisfactory demand on the part of the food industry.

POZSÁR, B. I.: It would perhaps be reasonable to fix the price of invert sugar at 80% of the price of saccharose (related to dry matter). The price of saccharose produced from sugar-beet is subsidized by the state, so it is not likely that the low price indicates economic efficiency.

RUFF, J.: I suggest fixing the price of liquid invert sugar obtained from maize at 20—30% lower than that of beet sugar, because

— invert sugar can be produced from maize all the year round, so its fixed asset demand is lower;

— there is no need for crystallization, drying and packing;

— its transportation even at a 70% concentration is cheaper, as it can be fully mechanized, and the labour demand is minimal;

— the transport cost of the basic material (maize) is only 20—25% of that for sugar-beet

Apart from this, the processes of dissolving and sometimes inversion before processing can be omitted; the impact of this on the costs will be felt directly by the user.

To sum up, the production of liquid invert sugar from maize is now opportune in Hungary as elsewhere. Invert sugar will be a strong rival, though not an enemy, to beet sugar. The two products are complementary to each other and offer a wider choice to the consumers.

SHMILLIÁR, M.: In a paper published in Zuckerindustrie 27, No. 1, January 1977 (pp. 42—43) Professor Wolfram (Institute of Agricultural Market Research and Economic Sociology, Bonn) compares starch sugar with beet sugar and analyses the price trends. In his summary he writes that according to his calculations syrups containing fructose are competitive with beet sugar. The production costs of starch sugar obtained from maize are 22 DM/q lower than those of beet sugar when calculated as white sugar equivalent. When calculating manufacturing costs the value of the by-products is a decisive factor. With the improvement of the processing technology the efficiency of extraction is expected to increase in the future, which again will result in a further cost reduction.

The price of liquid invert sugar obtained from maize should be lower than that of granulated beet sugar. According to American economists the price of liquid invert sugar must have a favourable effect, from the consumer's point of view, on the trend of sugar prices. The price of liquid invert sugar is influenced by the relatively cheap raw material and the simple processing technology. Its price should be fixed at about 80—85% of the current price for granulated sugar, since if it were higher both industrial and household utilization would be uneconomical.

SÓLYOM, L.: Compared to the state-subsidized wholesale price of beet sugar the manufacture of invert sugar should be profitable. Calculations made on the basis of dry matter content do not justify any substantial price difference between the two types of sugar.

SZÁNTÓ, S.: The price of 1 kg granulated sugar should be equal to that of 1.43 kg 70% liquid sugar. Liquid sugar should not be more expensive though possibly somewhat cheaper than that.

TYIHÁK, E.: It should be fixed at around 50%.

ZELLER, GY.: It is very difficult to suggest a retail price for invert sugar as a percentage of the price of beet sugar. This would require a knowledge of costs and of non-recurrent and continuous inputs. As the closest approximation I would suggest the following:

— The 70% dry matter content implies that the price of 1 kg liquid invert sugar should certainly not be higher than 70% of the price of (granulated) beet sugar.

— In order to gain a market the price of liquid invert sugar should be proportionately cheaper than the price of beet sugar. I have already mentioned a so-called "penetration" price; this aspect of price policy demands that the price of invert sugar should be fixed at 55—65% of the price for beet sugar, to make it appreciably cheaper. The problem is, however, that the initial price must be maintained for a considerable time; a comparatively flexible price policy cannot be interpreted in such a way as to apply a "penetration" price until a market is found and then, when the market seems to be stable, to raise the price, even if the price of beet sugar has not increased. A price stabilized for a long period is a basic requirement. Yet for a long period it is impossible to fix a price that does not ensure a reasonable profit above the production costs.

Thus, as I have said, if the production costs make it possible, the price of liquid invert sugar should be fixed at 55—65% of the price of granulated sugar, while the granulated form should cost somewhat (5—10%) less than beet sugar. The latter would, naturally, be produced only if the demand for liquid invert sugar remained below the production volume, or if the increasing demand for sugar could be more economically covered with granulated invert sugar than with beet sugar.

I am thinking here chiefly of the economic efficiency and possibilities of basic material production, also taking into consideration the profitability of manufacturing, in which the elimination of the seasonal character is an essential factor.

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LECTIONES

WORLD INTEGRATION IN GENETIC PROGRESS OF MODERN MILK AND BEEF PRODUCTION*

In the decades to come it seems that the most important target for animal production will be the raising of efficiency. The reason for this is the general unsatisfactory supply of the world population with food in general and animal protein in particular. It is a well-known fact that the transformation of vegetal energy, especially protein, into animal produce is burdened with a considerable loss. Hence, primarily in developing countries, farm animals which consume cereals often become the competitors of man. Food is also becoming a major political factor and its production together with the required energy has a decisive impact on the fundamental well-being of the world population. These may be the main reasons why the lowering of transformation losses is becoming of eminent interest, not only in animal husbandry but also in the whole world economy.

The fact that the most advantageous transformation in animal production is achieved by the high producing milk cow, renders this type of production of outstanding importance. This transformation coefficient reaches 35—40% as regards food energy as well as protein. It is, however, very unsatisfactory that it is in this very branch of animal husbandry that the level of production is relatively the lowest, especially compared with the biologically possible limits. A glance at Table 1, which represents the average production and the present biological limits of the producing capacity of some important species in Hungary, shows that large differences exist.

Table 1

Some data on average production in Hungary and the maximum attained

	Milk production per cow		Beef (bulls)		Pork production		Egg production per hen	
	Milk		Daily gain		Daily gain		Eggs	%
	kg	%	g	%	g/day	%		
National average	2 700	100	1000	100	450	100	240	100
Average of high producing farms	5 500	204	1300	130	650	144	270	113
Maximum individual	25 247	935	2000	200	1167	259	367	153

* Lecture delivered at the plenary session of the III. All Union Genetic Congress in Leningrad, 1977.

It may well be noted that the limits of milk production with the technology in use at present surpass 3 to 6 times the respective production of other intensively producing species. It is hardly conceivable, at least at present, that a laying hen could produce 800—900 eggs per year, or that pigs could have a daily growth rate of 2000—3000 g. In contrast to this, cows producing 500—900 kg butterfat or 15,000—25,000 kg of milk per year can do this without having to encounter a biological or physiological break-through.

I believe that even supposing a production of 200—250 kg butterfat or about 6000 kg of milk per year in specialized milk producing units we are on the threshold of a very great development in milk and beef production. This development will be achieved by an integration into the world's genetic progress. Today in animal breeding there is a world-wide cooperation, in which even the largest countries cannot keep their standard at a world level in all branches of animal production. We must always use the genetic progress achieved somewhere in the world, and take advantage of the genetic progress of the big gene-reserves. These gene-reserves cannot be easily translocated, because the high genetic pressure can only be applied within relatively large populations with a high standard of selection and production. The transfer of such populations, in contrast to plant breeding, is impracticable. Competition is also very keen, and it takes very up-to-date genetic work with a large basis of breeding stock to develop the level of any hybrid or pure breed of a certain type. However, the continually increasing number of types and varieties required partly by the various technologies of production and the genetic-environmental interactions prevent breeding from becoming the monopoly of certain countries.

A good example of the world-wide integration in cattle breeding today is the development of the USA-Canadian Holstein, which had an average production of 5900 kg of milk in Hungary in the year 1976. If we extrapolate the genetic trend of this development, together with the increase in production of some well-managed breeds, e.g. Danish Jersey and others, then we can state that the annual development amounts to a production increase of about 1—2%. This means that the USA-Canadian Holstein may reach an average production of 8500 kg of milk within 20 years. Today we face a situation where the European countries could not modernize their milk producing cow population within a short time without making use of the USA-Canadian Holstein. At the same time the USA and Canada could not develop their beef producing cattle populations without French and Central European beef and dual purpose cattle breeds. A very similar trend can be registered in various branches of animal husbandry. Apart from more efficient technologies, this world integration is largely helped by the new achievements in biotechnical research and facilities, such as the deep freezing of semen, the practice of embryo transplantation, transport facilities for breeding stocks, etc.

We are gradually coming to a time, when we cannot afford not to make use of this continuous genetic development. With only a modest 1% progress per year, the lagging behind of possibilities within 20 years would reach 20%, which is a loss that cannot be tolerated by any well managed economy.

In the milk and beef production of today, even on a restricted area, at least 5 different types of population or breed are necessary to attain optimal efficiency. The reason for this is that in cattle production not only is efficient milk production of importance, but also an optimal level of fertility (calf production) and beef production. This, however, is a very contradictory procedure, since the efficiency of calf production and a high capacity of growth rate represent a biological and physiological incompatibility. The type of cow which will produce a weaned calf efficiently must be early maturing, of moderate body weight, of good mothering faculties and an easy calver. For fattening, on the other hand, we need a late maturing, large type of animal with a considerable capacity for growth rate. These characteristics are incompatible, so they cannot be combined within one population and can only be made use of by cross-breeding.

Cross-breeding is gaining ground all over the world, in the first place because a population or breed can be enriched by qualities which could only be attained over a large number of generations with the aid of pure breeding. The second reason for the great advance in cross-breeding is the growing importance, especially in large scale enterprises, of the utilization of the heterosis effect. Table 2 (by Preston—Willis) gives a survey of the world literature on the

Table 2

Heterosis affecting mostly beef production, in %
(Preston — Willis 1974)

Quality	No. of authors	Heterosis %		
		extreme	values	average
Birth %	24	— 2	+43	+12.3
Birth weight	24	—20	+23	+ 4.8
Vigour	24	— 6	+44	+ 5.8
Growth rate from birth to weaning	18	0	+14	+ 6.7
Weaning weight	42	— 3	+23	+ 7.5
Growth rate from weaning to slaughter	32	— 5	+25	+ 5.0
Feed conversion	8	— 6	+ 7	+ 2.0

different qualities which are affected by heterosis, and of the accumulative effect of heterozygosity. The superiority is especially striking in the qualities affecting fertility and stress resistency, which are so very important in large scale enterprises. Further to these aspects of cross-breeding in the last years, a new term is coming into use, namely "profit-heterosis", which I should like to change to "economic- or type-heterosis". This type of heterosis means the optimalization of the different population-types, such as the efficient milk producing cow with excellent constitutional qualities and fertility, the single suckler cow of small statue as the efficient calf-producer, the type transmitting a high growth rate for economic fattening, the male lines assuring easy calving for heifers, etc. All these different types represent specialized morphological characters which are more or less incompatible. This full utilization of heterosis may result in an overall superiority of production, which may be estimated at about 20%.

The future in cattle breeding requires a different genetic organization than that in practice today. On the one hand we need a number of pure bred cattle types and populations, and on the other, milk and beef production should be carried out mostly using cross-bred populations. The research work on large populations by R. C. Cartwright, E. P. Cunningham, H. P. Donald, A. Horn et al., M. M. Lebedev, J. H. Lerner, G. Schönmuth, H. Skjervold, Weber etc. all underline these statements. The breed therefore determines the system of production less and less. Intensive genetic work is needed within the pure breeds, which have to produce the necessary number of sires of top quality. Considering the rapid progress in the genetic structure of pure breeds and the various types necessary for the synthesis of populations meant for production, a vast international cooperation is necessary, which can keep pace with the world's genetic progress within all pure breeds. Fig. 1 shows the new organization of milk and beef production, which is gradually being introduced in Hungary on a large-scale experimental basis. The system also integrates the maximum utilization of the heterosis effect, not only from a classical point of view, but also by an optimal utilization of the type differences in various populations. This breeding system synthesizes the advance in selection achieved on

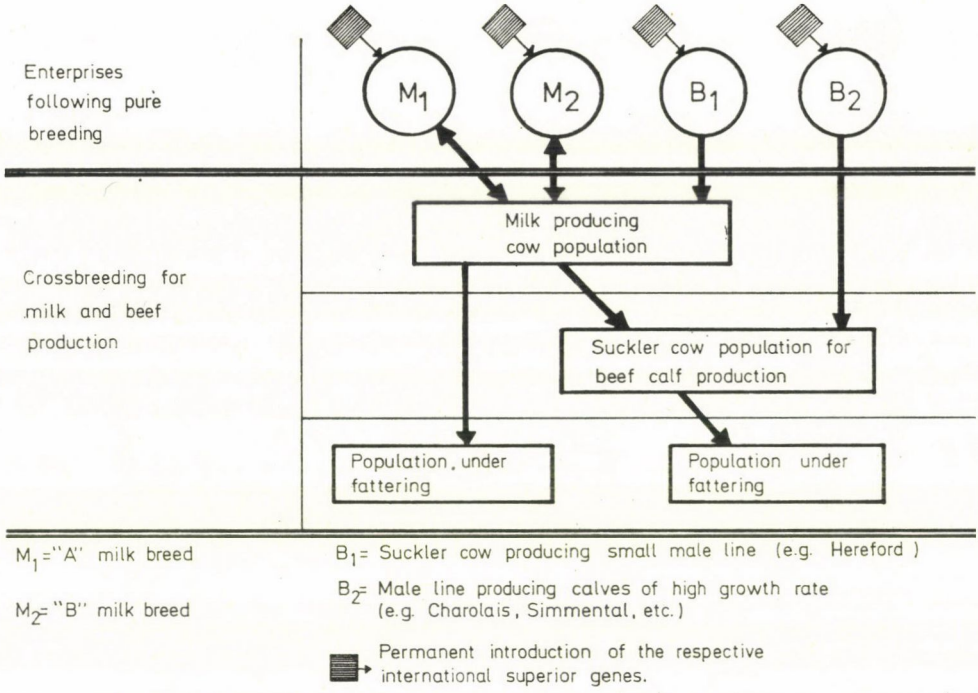

Fig. 1. **The role of different cattle types in modern production**

Table 3

The most important qualities of the Canadian Holstein and Danish Jersey breeds according to progeny test results in the first lactation (CC test) (A. Dunay — S. Bozó)

		Holstein*	Danish Jersey**
Milk production	kg	4936	3194
Milk fat	kg	184	195
Milk fat	%	3.73	6.11
Protein	kg	159	139
Protein	%	3.23	4.35
Age at first calving	month	29.3	25.0
Weight at first calving	kg	560	359
FCM/100 kg live weight	kg	845	1170
Udder quality		+++	++++
Milkability		++++	++++
Reproducing ability		++	++++
Useful span of life		++	++++

* = 1833 bulls 115,608 daughters (1974, Canada).
** = 37 bulls 2624 daughters (1974, Denmark).
——— = The breed ranks first at world level.
++ = medium; +++ = good; ++++ = excellent.

Table 4

Genetic prognosis for the production of the crossbreds
(A. Dunay — S. Bozó)

Generation	Genetic structure						Expected production of cows									
	"A" population			"B" population			"A" population					"B" population				
	HF	H-f	J	HF	H-f	J	Milk, kg	Fat, kg	Fat, %	Prot. %	3.6% corr. milk	Milk, kg	Fat, kg	Fat, %	Prot. %	3.6% corr. milk
I	50.0	0	50.0	50.0	50.0	0	3880	192	4.96	3.80	5333	5141	193	3.75	3.25	5361
II	25.0	25.0	50.0	25.0	50.0	25.0	4870	239	4.90	3.78	6639	5514	237	4.30	3.50	6583
III	12.5	25.0	62.5	12.5	62.5	25.0	5016	260	5.18	3.91	7222	5987	256	4.27	3.49	7111
IV	6.25	31.25	62.5	6.25	62.5	31.25	5359	277	5.16	3.89	7694	6185	272	4.40	3.55	7555
V	3.1	31.3	65.6	3.1	65.6	31.3	5508	288	5.23	3.92	8002	6437	283	4.40	3.55	7861

Remarks: The genetic calculation is based on the following data:
Holstein-friesian (H-f) 6514 milk, kg — 236 fat, kg — 3.63 fat, % — 3.20 protein, %.
Danish Jersey (Jx) 4133 milk, kg — 250 fat, kg — 6.06 fat, % — 4.30 protein, %.
Hungarian Fleckvieh (HF) 3186 milk, kg — 123 fat, kg — 3.86 fat, % — 3.30 protein, %.
Effect of heterosis: generations I and II: 6% in milk production; from generation III: 4% in milk production.
Genetic progress: 2.4% in milk production.

a high international, possibly world level in pure breeding. In this respect there should also be much greater international cooperation in the Comecon countries.

An example of two milk producing breeds may illustrate the genetic possibilities of combining the parameters of two breeds by using a criss-cross breeding system. Table 3 shows the producing ability and the qualities of the Canadian Holstein and the Danish Jersey breeds.

Table 4 shows the genetic prognosis for the two cross-bred populations starting from Hungarian Fleckvieh (HF).

In genetics the term "genetic engineering" is often used today. This is generally understood to mean the technical transfer of certain genes into other genotypes. In my opinion, however, the term "genetic engineering" should be applied in cattle production to a different conception. It should mean an optimal use of the type differences incorporated in various breeds and the effect of cross-breeding. In this conception breeds are no longer the objects of production, but the building stones of the production systems. This aspect of breeding demands a different outlook from breeders and breed organizations, as well as on the part of the governmental officials responsible for production. I am convinced that the principles outlined will play a decisive role in future cattle production and that a spectacular new trend in milk and beef production with very fast all-round development may be expected in the next decades. We are faced with a new production strategy, which needs new aspects and an open acceptance of the new facts of genetic development in the world. We can no longer afford to ignore or not to utilize directly whatever has been discovered or achieved in breeding anywhere in the world.

A. Horn
Department of Animal Husnbandry,
University of Veterinary Science,
H-1077 Budapest
Rottenbiller u. 23—25.

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CHRONICA

HOW CAN FOOD PRODUCTION BE DOUBLED IN QUANTITY AND IMPROVED IN QUALITY TO MEET HUMAN NUTRITIONAL NEEDS BY THE YEAR 2000?

At a scientific meeting of the United Nations University (UNU) in Stockholm in mid-March 1977, at UNU's initiative, the foreign secretary of the Royal Swedish Academy of Sciences, O. Tandberg, raised the idea of an international scientific workshop on agricultural production and human nutrition, which it was hoped might be held at Martonvásár in late spring 1978 with the participation of invited specialists from the five Nordic countries and the eight socialist countries of Central and Eastern Europe, together with representatives from the relevant international organisations.

The idea of the workshop was adopted by the Hungarian Academy of Sciences and the Royal Swedish Academy of Sciences and was included in the cooperation agreement. An 8-man organising committee consisting of representatives from the two academies was set up, and this committee decided on the subject and programme of the workshop, planned and organised the workshop itself and is now arranging for the publication of the proceedings.

Right from the beginning the organisers looked forward to the workshop with what were felt to be justifiably great expectations. There were several reasons for this. First, the subject matter promised to be interesting, as it was designed to produce an exchange of ideas and a clash of views between agriculturalists and nutritionists. But our expectations were also raised by the fact that, due to the different historical and cultural backgrounds and the unequal economic development of the peoples of Northern Europe and of Central and Eastern Europe, specialists from the two regions have different attitudes to questions vitally concerning humanity. The basic concept of the workshop, "How can food production be doubled in quantity and improved in quality to meet human nutritional needs by the year 2000?" was intended to give some sort of unity to this diversity of ideas, but even before the workshop began, when we were deciding on the subjects of the papers, it became obvious that we were not completely unanimous in our interpretation of this either. This all increased our sense of anticipation and gave us renewed strength in overcoming all the difficulties involved in the organisation, even at moments when it looked, as it did at one time, as though all was in vain.

The Hungarian Academy of Sciences invited two specialists, one agriculturalist and one nutritionist, from each of the socialist countries of Central and Eastern Europe (Bulgaria, Czechoslovakia, East Germany, Hungary, Poland, Romania, the Soviet Union and Yugoslavia) to attend the workshop, while the Royal Swedish Academy of Sciences extended invitations to two specialists in the same fields from each of the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden).

Invitations were also sent to five agencies of the United Nations Organisation, namely to UNU, which initiated the workshop, and to FAO (Food and Agricultural Organisation),

ICSU (International Council of Scientific Unions), UNESCO (Educational, Scientific and Cultural Organisation) and WHO (World Health Organisation) to send observers to the workshop.

Twenty-five specialists from the eight Central and Eastern European countries (including 16 Hungarians) and 11 specialists from the five Nordic countries attended the workshop, together with one observer from each of the UN agencies.

In accordance with the original plans, the workshop was held at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, from June 5—9th 1978. The official language was English, but two English-Russian interpreters were employed to assist one of the foreign participants.

After an opening address by János Szentágothai, academician, president of the Hungarian Academy of Sciences, brief welcoming speeches were also made by the representatives of the international organisations, who underlined the importance of the workshop and of its subject matter. These were followed immediately by the scientific papers.

One session was held each day. The main topic of the first session was "The Nutritional Needs of Man". Under the chairmanship of A. Ahlström (Finland) two papers were presented, "Human nutritional needs — general considerations" by B. Isaksson (Sweden) and "Human nutritional needs with special reference to essential nutrients" by H. Haenel (East Germany).

The second session, under the chairmanship of M. Volgarev (USSR), dealt with "Malnutrition" and again there were two papers, "Problems of overnourishment" by K. Norum (Norway) and "Problems of undernourishment" by R. Buzina (Yugoslavia).

The chairman of the third session was B. Sigurbjörnsson (Iceland) and the main subject was "Plant Production". There were three papers in the programme, first "Management potentials for crop improvements" by N. Turbin (USSR), then "Genetic potentials for improved crop yield" by J. Mac Key (Sweden) and finally "Genetic potentials for improved crop quality" by L. Munck (Denmark).

The fourth session was chaired by Gy. Pethes (Hungary) and was devoted to "Animal Production". Papers were presented by B. Eggum (Denmark) on "Criteria for optimising animal feeding" and by F. Fekete—T. Ferenczi on "Optimising animal production towards human nutritional needs".

The papers in the third session were each of half an hour's duration and those in the other sessions 45 minutes. It reflects to the credit of the speakers that none of them overstepped the time at their disposal, despite the fact that almost without exception they delivered their lectures without reading from their notes. It is perhaps worth noting that of the 20 hours available for the sessions only 6 hours were taken up by the presentation of the papers, leaving 14 hours for informal discussion. The only time restriction mentioned in the general information section of the programme was that no contribution should last more than 10 minutes. To the satisfaction of all present, this restriction was also complied with. At the same time nobody had to be pushed into speaking; on the contrary, the chairmen always had a list of several delegates who were waiting to speak.

Any comments on the success of the workshop are bound to be one-sided and inadequate coming, for example, from the pen of a geneticist, but this is only to be expected. Human nutrition is designed to satisfy various needs, namely, 1) the energy requirements of the metabolism and of movement (work), 2) the materials required for the build up, maintenance and replacement of cells, tissues and organs and 3) the materials required for the construction of regulating substances, enzymes and hormones.

The organism is not capable of synthesising certain molecules (certain amino and fatty acids, vitamins); on the other hand, foods which provide energy do not contain certain mineral elements. Together, these molecules and mineral elements make up the group of essential nutrients. Water, which the organism is unable to synthesise in the quantities required if it is to function properly, should also be included in this group.

However strange it may seem, we do not feel hunger for essential nutrients, except for water, for which we feel thirst. We only feel hunger for energy, that is, for quantity, not for quality. In other words, we cannot rely exclusively on our instincts in choosing our food or in directing agricultural production, which is our primary source of food. We require in addition a knowledge of the composition and essential nutrient content of various foods and of their anti-nutritional and toxic substances. Apart from this it is indispensable to know what demand there is for various foods, and we must also know how the human organism digests, absorbs and converts nutrients, what factors influence these processes, how our everyday foods affect each other, etc. And if we really do have the possibility of doubling food production by the year 2000, it is imperative that this increase should promote and not obstruct healthy nutrition and should compensate for the imbalance characteristic of our diet.

The greatest health problem of our time is malnutrition, the results of which are undernourishment and overnourishment. These have similar consequences: a shortening of the expected life-span, increased susceptibility to diseases, and reduced working capacity. Overnourishment, the greatest plague of modern times, affects almost as many people as undernourishment. Despite the fact that undernourishment is mainly characteristic of the populations of developing countries and overnourishment of those of industrialised countries, this certainly does not mean that undernourishment has been eliminated in our countries, even though we never see starving people. The truth of the matter is, that the effect on our diet of increased production, the refinement and development of products, and trade and price policies can lead to both overnourishment and qualitative undernourishment.

From the beginning of the century to the end of the sixties the consumption of cereals declined in the developed, industrialised societies, e.g. in Sweden, and was replaced by fat and sugars, so apart from fat-soluble vitamins and unsaturated fatty acids the consumption of essential nutrients decreased rather than increased, i.e. the dietary change was basically the opposite of desirable. In other words, in industrialised countries the cause of undernourishment, except in certain population groups, is not lack of food, but rather the low nutrient density of the food (= essential nutrients per unit of energy), which can result primarily in iron-deficiency anaemia, but also in other mineral and possibly vitamin deficiencies. Apart from this the diet is poor in fibre (celluloses, hemicelluloses, lignins and pectins), which may raise the frequency of intestinal diseases and may even produce gastro-intestinal cancers.

During the sixties the role of protein was overemphasised, while in the first half of the seventies the protein requirement was underestimated and greater significance was attached to the fulfilment of the energy requirement. Nowadays, nutrition science acknowledges the importance of both protein and energy, and work on a more realistic estimation of the protein requirement continues to be an important field of research.

Thus, the ideal composition for human nutrition is as follows: A satisfactory quality and quantity of protein, sufficient fat to carry the essential unsaturated fatty acids and fat-soluble vitamins and to reduce the mass of food consumed, sufficient carbohydrate to satisfy the remaining energy requirement, and in addition vitamins, minerals and fibre. 12—14% of the total energy should stem from proteins, 30—35% from fats and 53—57% from carbohydrates. Only for nursing mothers should the energy derived from protein reach 15% of the total energy.

Man's genetic and physiological (metabolic) adaptability can in fact suit him for very different diets. Eskimos, for example, live and thrive on a diet containing 25—30% protein energy. But high protein consumption generally shortens life expectancy and increases the toxicity of certain chemicals, a fact which should, before it is too late, be brought to the attention of those young people who, in their desire to slim at any price, live on an almost exclusively protein (lean meat and cheese) diet. The virtually complete elimination of bread from these slimming diets may reduce to a minimum the consumption of nutrients rich in fibre, since

bread, particularly wholemeal bread, contains large quantities of fibre, which is of great importance as it promotes and accelerates the movement of the bowels. This is why there is now a campaign in Sweden to increase the consumption of bread. This is noteworthy even when we consider that the annual per capita cereal consumption in Sweden in the mid-seventies was only just over 50 kg, which is only about 40% of the Hungarian consumption, but still a lot more than the complete lack of cereals in the slimming diet!

One of the turning points in the evolution of dietary habits usually coincides historically with the industrial revolution. This is the period when the consumption of cereals as staple food begins to decline and foods consisting of "empty calories" (fats and sugars) take their place. At the same time, due to the greater consumption of animal products and of fruit and vegetables, the diet becomes more varied. To some extent we are now witnessing this process in Hungary and it would be a good thing if we could avoid repeating the mistakes made in the developed industrialised countries. As one of the Nordic nutrition experts said at the workshop, we should learn by the mistakes of others and not add unnecessarily to the number of people suffering from degenerative diseases, such as coronary and circulation diseases or diabetes, since the number of cases of these diseases is regrettably quite high enough already.

When debating the possibility of doubling plant and animal production, the delegates were unanimous in their opinion that agricultural production is generally far from achieving what has been demonstrated to be its optimum performance. Due to man's contribution the energy which passes through the green plant, the primary source of organic matter, as if it were being forced through the eye of a needle, is capable of producing really large yields, e.g. bumper grain crops of over 10 tons/ha have been recorded for wheat, and even higher yields for maize. But this is still far from the upper limit calculated on the basis of the solar energy reaching the surface of the Earth, the efficiency of photosynthesis and the energy balance for the conversion of inorganic to organic matter, which is equivalent to a grain yield of 35—40 tons/ha for wheat.

For hundreds of years plant production increased very slowly, if at all, until the time when plant breeding and modern plant production, especially intensive fertilisation, resulted in spectacular advances, which are justly attributed to a number of outstanding scientific achievements (heterosis, large doses of well-balanced nitrogen fertilisation combined with denser plant stands and successful pest control, etc.). A good example of this is the Hungarian wheat and maize yields, which have increased 2 1/2 times over the last twenty years, as we mentioned at the workshop, adding, however, that the Hungarian example is by no means unique on a world scale. It is enough to refer to France or Mexico, where the wheat yields showed similar increases during the same period, not to mention maize production in the USA, which underwent a fantastic development in the middle third of the century.

By breeding plants and animals and developing their nutrition or feeding, we are able to modify those products which are suitable for human consumption and adapt them to satisfy man's requirements in amazing ways, and we have still not reached the limits. Geneticists have produced dwarf versions of cereals and other cultivated plants, legumes free of toxic substances, tomato plants producing square fruit, they have learnt to synchronise flowering to suit various purposes and have increased the fat content of milk, though if necessary this could be reduced again, or cows could be bred whose milk suited the requirements of babies rather than those of calves, etc., etc.

All in all the final statements and proposals made in the last paragraph of the general conclusion adopted at the close of the workshop seem to be well-grounded:

"The technical capability exists today for producing enough food of such a quality that all of mankind could be supplied with an adequate diet. The constraints are low purchasing power, the limited application of existing knowledge, ignorance about sound food practices

and lack of capital for obtaining the necessary agricultural inputs. Development efforts must primarily be aimed at alleviating or circumventing these constraints."

The workshop sessions were combined with demonstrations and institute visits, and with professional and cultural tours. The professional programme included the Martonvásár institute, particularly the phytotron, the wheat nurseries and the seed processing unit at our experimental farm, and visits to the Institute of Nutrition, Budapest, and the Bábolna Agricultural Combinate. The cultural programme comprised visits to Buda Castle, including the National Gallery, to Pannonhalma Abbey to see the religious and cultural relics, and to an opera performance at the Budapest Opera House.

On my way home from our extremely interesting visit to Bábolna and Pannonhalma on the final day of the workshop an idea kept running through my mind, which I included that evening in a toast at the farewell dinner given by the Secretary General of the Hungarian Academy of Sciences: "We must be careful never to clip the wings of those who are capable of flying higher than the rest, for the fate of humanity depends on those wings."

Now, several weeks after the workshop, having read the letters of appreciation sent by many participants, I feel justified in adding that the spirit and atmosphere of this meeting also did good service to the development of science and of human relations, because it created a free forum for the exchange of ideas.

S. Rajki

Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 28 (1—2), pp. 265—274 (1979)

RECENSIONES

Annales de la Faculté des Sciences. Université de Dakar. Dakar, 1974, 27.

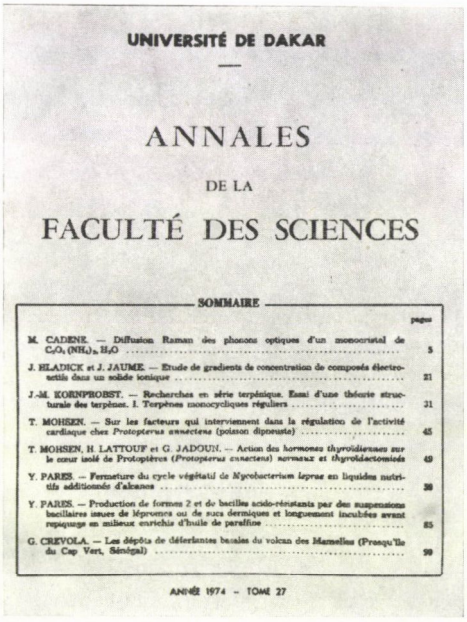
The 1974 year-book (tome 27) published in French by the Faculty of Sciences of the University of Dakar, a prominent university of the "Third World", contains 8 papers covering 99 pages.

The subjects of the papers are taken from various fields of the natural sciences.

M. Cadene's work (Diffusion Raman des phonons optiques d'un monocristal de $C_2O_4(NH_4)_2\ H_2O$) falls within the scope of crystal chemistry. The author studies the Raman scattering spectra of ammonium oxalate monocrystals in polarized light in the spectral range of 30—4000 cm^{-1}. The results of the examinations confirm that the structure determined by Roentgen-diffraction measurements is correct.

The second paper (J. Hladick et J. Jaume: Etude de gradients de concentration de composés électroactifs dans un solide ionique) is of physico-chemical character, as may be seen from the title. The authors study the formation of electro-active compounds in solid electrolytes by means of chronoamperometry. The theoretical amperometric curve is calculated by means of a double Laplace transformation. The points on the curve coincide with the calculated values.

J. M. Kornprobst, author of the third paper (Recherches en série terpénique. Essai d'une théorie structurale des terpènes. I. Terpènes monocycliques réguliers), presents a paper on theoretical organic chemistry. On the basis of the results obtained in the course



UNIVERSITÉ DE DAKAR

ANNALES

DE LA

FACULTÉ DES SCIENCES

SOMMAIRE

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of theoretical investigations into the structures of terpenes he establishes that a generalization of the terpene structure opens up great possibilities for research in synthesizing neo-terpenes similar to natural substances. A detailed study of possible ring structures formed from isoprenic units, together with research concerning monocyclic skeletons, led to the notion of a "basic ring". The number of basic rings is 5, in a skeleton of a given size.

The subject of the fourth paper is taken from the field of animal physiology [T. Mohsen: Sur les facteurs qui interviennent dans la régulation de l'activité cardiaque chez

Protopterus annectens (poisson dipneuste)]. Studies on various factors influencing the cardiac activity in dipnoan fishes seem to suggest that in the biological cycle of lungfish at the beginning of the enkystment phase the diminution of the thyroid and cholinesterase activities in compensated by an unknown factor, probably of an adrenergic nature.

The paper written by the above author with two co-workers. H. Lattouf and G. Jadoun, deals with a subject in the same field [Action des hormones thyroïdiennes sur le cœur isolé de Protoptéres (Protopterus annectens) normaux and thyroïdectomisés]. The authors demonstrate the positive chronotropic action of thyroid hormones (T_3 and T_4) on an isolated heart. This action was limited to the aquatic phase of the animal's life cycle.

Y. Pares presents two papers on microbiological subjects. One of them (Fermeture du cycle végétatif de Mycobacterium leprae en liquides nutritifs additionnés d'alcanes) discusses the behaviour of Form 2 of Mycobacterium leprae in various media supplemented with saturated open-chain hydrocarbons. In such culture media the bacteria assumed an acid fast state.

The other paper by Y. Pares deals with the survival ability of the microorganisms mentioned. Bacillary suspensions were incubated for twenty four to forty two months in various liquid media without any cell division occurring. The nutritive media in the test-tubes remained as clear as water. After the long period of incubation the microorganisms were inoculated onto media supplemented with paraffin carbohydrate. The experiment gave strong evidence of the survival of the microorganisms, for in spite of the long incubation period, in media containing hydrocarbons BAAR 2 forms or mixed forms developed from the cultures.

The last paper in the year-book is a geological study [G. Crevola: Les dépots de déferlantes basales du volcan des Mammelles (Presqu'île du Cap Vert, Sénégal)]. The author gives an account of the structures of tuffs produced by the quaternary activity of the Mammelles volcano, and draws conclusions from them on the types of volcanic explosion.

The year-book also contains a list of contents of the earlier year-books, published since 1954.

L. GÁSPÁR

Studia Phytologica in Honorem Jubilantis A. O. Horvát (1977).

Professor A. O. Horvát, world-famous researcher of the flora and comparative geobotany of the Mecsek hills in southern Hungary, author of numerous monographs and of works on forest-typology, environmental protection expert of the Pécs Academic Committee, celebrated his 70th birthday on 6th March 1977.

On the occasion of his birthday he was warmly greeted by his pupils and friends, who published a collection of papers as a token of their esteem. The work consists of 26 monographs written by Horvát's foreign friends in German, French, English and Russian and published in alphabetic order of the authors' names. The costs of publication were met by the political and scientific leaders of the county of Baranya and the city of Pécs.

Aichinger, E. (Austria): describes the hornbeam-oak forests of the Alps and Karawanka, which are similar to the deciduous forests of the Mecsek, which the author once roamed together with Professor Horvát.

Archiloque, A.—Borel, L. (France): The authors present a geobotanical view of the Jurassic and cretaceous juniper groves in the South-Western Alps, in which they wandered with Professor Horvát.

De Bolós, O. (Spain): As a Catalan, the author presents his native land from the Pyrenees to Valencia, a zone with about 500 km of coastline, together with the Balearic Isles. Particular emphasis is laid on those flora components of the Alps and the Carpathians which are common to the vegetation examined.

Dancau, B. (German Federal Republic): The author uses phytogeographic data to prove that the basis of plant production is

regional landscape planning, following a reconstruction of the original and potential vegetation.

Fukarek, F. (German Democratic Republic): The author has studied the occurrence of Reseda lutea on the shores of the North Sea on excursions made with his friend.

Fukarek, P. (Yugoslavia): From vegetation and forest-typology studies the author has drawn the conclusion that the beeches found in the *Pannonicum* do not belong to the Fagion illyricum species but to Fagion circumpannonicum, which he himself introduced. This phytocenological classification agrees with the opinions of Professors Horvát and Pócs, who place *Transdanubia* with the exception of the Zákány district in the *Pannonicum* and not in the *Illyricum*.

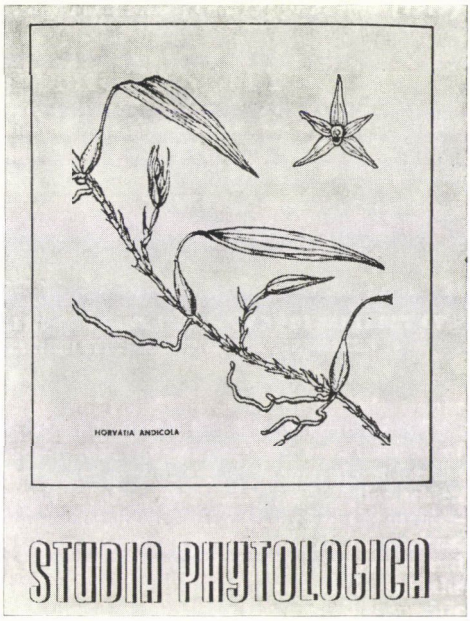
Garay, L. (United States): a student of Professor Horvát, born in Hosszúhetény (Hungary). As his contribution to this volume he describes a new orchid genus occurring in Ecuador (*Horvátia* Garay gen.) and reports on a new species [*Horvátia andicola* (Garay)].

Georgen, J.—Schmithüsen, J.—Schreiber, H. (German Federal Republic): The authors have carried out microclimatic studies (by measuring the soil temperature, air temperature, intensity of evaporation, air humidity) in the Bükk hills in North-eastern Hungary, in the area of Felsőtárkány, Bánya and Nagymező, together with floristic, phytogeographic and zoocenological surveys; the occurrence of butterflies was studied in great detail.

Grandtner, M. M. (Canada): presents the coastal association of Cakiletum edentulae in the Forillon national park (Quebec).

Hundt, R. (German Democratic Republic): compares the onion-couch meadows of the Mecsek, described by Professor Horvát, to meadows in East Germany, and to those in England which he studied together with Professor Horvát, on the basis of phytogeographic and cenological characters, with particular emphasis on the flora components.

Hübl, E. (Austria): demonstrates central and eastern submediterranean geoelements in the West Pannonian region which he studied with Professor Horvát. He thus agrees with Professor Horvát's opinion of the flora elements of the region, which are incorrectly regarded as *Illyricum* or *Pannonicum* taxa.



Knapp, R. (German Federal Republic): The world-famous phytocenologist has prepared a comparative phytogeographic and forest-typological work on the subcontinental xerothermic *Potentillo-Quercetum* forests and mixed oak forests, which falls within Professor Horvát's main sphere of interest.

Linskens, H. F.—van Vroonhoven, C.—Ljzerman, E. (Holland): On the basis of original vegetation studies the authors survey and quantitatively evaluate the typology of the wind-caused distortions of trees, and interpret the mechanism of wind tolerance on the basis of the development of the root system.

Löve, Á. (United States): On the basis of theoretical considerations the author analyses the role of the gene pool in the human environment, and the importance of gene preservation in the protection of nature, a subject related with Professor Horvát's work on the protection of endemic species.

Neuhäusl, R.—Neuhäuslová-Novotná, Z. (Czechoslovakia): The authors describe the

Czechoslovakian oak-woods corresponding to the dry oak-woods of the Mecsek, including a phytogeographic characterization.

Numata, M. (Japan): presents the deciduous forests of Japan, in particular the beech-groves, and compares them with the beech-groves of the Mecsek on the basis of Professor Horvát's hand-book.

Passarge, H. (German Democratic Republic): presents the species groups and syntaxonomy of *Potentillo-Quercetum* cenoses occurring in the German Democratic Republic, comparing them with the associations found in the Mecsek.

Pietsch, W. (German Democratic Republic): The occurrence of Aldrovanda on Lake Baláta (Hungary) and ecological and cenological descriptions of the species are compared to biotopes found in other countries. The author studied the cenology of Lake Baláta with Professor Horvát.

Pignatti-Wikus, E.—Pignatti, S. (Italy): The authors have studied the *Alysso-Sedetum* plant cenoses in the serpentine soils of the North Appenines south of Pavia, together with numerous endemic species.

Raus, D. (Yugoslavia): The author gives a richly illustrated description of the Slavonian *Carpino betuli—Quercetum roburis* forest type, and compares it with the Slavonian oak forests planted in Hungary.

Stojkó, S. (Soviet Union): gives a phytogeographical, phylocenogenetic, genetic and ecological description of the forests in Carpathian Ukraine, and publishes data on their phytomass production, underlining modern aspects of forest protection.

Tomaselli, R. (Italy): The author relates the phytogeographic characters of mediterranean and submediterranean elements in the Mecsek to the flora elements forming forests in the Mediterraneum.

Trepp, W. (Switzerland): describes the plant cenoses of fir-woods at Engadin, a dry growing site in the Graumünden nature reserve.

Wedelberger, G. (Austria): gives a description of the plant cenoses of solonetz and solonchak soils on the Austrian side of Lake Fertő.

Zukrigl, K. (Austria): The author deals with the plant cenoses of the Weinviertel island forests, and emphasizes that the turkey oak forests are artificially planted there, just as they are in the Mecsek.

The front page of the volume is decorated with the new genus and new species of *Horvátia andicola* (Garay) described by the world-famous orchideologist of Harvard University, Cambridge, Mass. (USA) with the words of a respectful student: "Professor Horvát gave botany to me, and at the same time gave me to botany. The help and advice I received from him has been of service to me all my life !"

The forest-typological aspects discussed in the book go beyond the interests of geobotanists and cenologists, and supply useful information for practical foresters as well.

B. I. Pozsár

Boletin de Reseñas, Serie: Ganaderie, 1976, *3*, 7, 8, 9—10; 1977, *4*, 1

Donald Calzadilla Dodd: Algunos aspectos sobre la importancia del intervalo entre partos en la produccion de leche (Some aspects of calving intervals of importance in milk production), (1976, *3*, 7).

On the basis of 96 literary references the efficiency of reproductivity, and the anatomical, pathological and physiological causes of low fertility are discussed. Factors influencing the fertility are also presented.

Milk has been known since ancient times as a nutrient of great value. Milk and milk products have high protein, calcium, phosphorus and vitamin contents. This is the reason for genetic, environmental and economic studies aimed at increasing the volume of milk production. Although milk production has assumed an industrial character, the performance of the animals has not yet reached a maximum on a world scale.

The greatest problem is represented by the low rate of conception. By using artificial insemination losses caused by male sterility can be reduced.

The author presents data of efficiency

calculations from dairy farms where the rate of fertility is low. The consideration of the calving interval is particularly suitable for such analyses.

Efficiency of fertility: sterility and subfertility, which may be caused partly by defective spermatogenesis in the bull, have become frequent in some dairy farms. When reproductivity is not satisfactory the cows have to be subjected to individual examination.

The most frequent hereditary anatomical and pathological causes are anomalies in the gonades and the abnormal nature of the spermatozoa. Some authors have studied the role of morphological defects including persisting hymen, insufficient development of the genital tract, double necked uterus, unicornous uterus, and underdeveloped ovaries. In the case of bulls the causes may be insufficient development of the testes, hernia, adhesion, and underdevelopment of the penis.

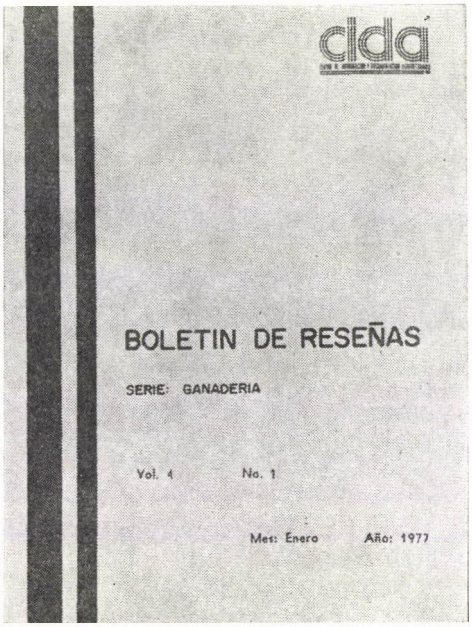
Pathological processes: brucellosis, vibrosis, trichomonadosis, leptospirosis and other non-specific infections.

Of the physiological causes, disorders of the endocrine system leading to irregularities in the oestrus cycle or to anoestrus may occur.

Methods of keeping and feeding are closely related with the efficiency of fertility, while milk production is dependent on the activity of fertility, as frequent calving naturally increases the volume of milk produced during the life of a cow.

Interval between calvings: the production of milk begins immediately after calving, but during the lactation period the production is influenced by various factors. The dry period following the lactation is extremely important from the point of view of preparation.

The frequency of pregnancy fundamentally influences the life performance. According to the evidence of reliable data cows calving every 12 months achieve a higher daily output compared to animals with larger calving intervals. The optimum 12 month interval cannot be realized in practice, usually owing to troubles arising from the first calving. In the case of prolonged intervals milk production may be higher in a given lactation period,



but the annual output remains below average.

The period of preparation: the time from calving to the next conception is highly important. It is generally accepted that enough time should be left for the regression of the genital apparatus before the new pregnancy occurs, and this is confirmed by the results of examinations in 1398 cases of calving. From the data the author draws the conclusion that higher productivity can be attained in the first and second lactation period if the preparatory period is somewhat longer.

Lactation: the author outlines the physiology of lactation. After pregnancy has begun the larger milk-ducts branch off. In the fourth and fifth months the lobuli of the lactiferous glands are sufficiently differentiated, and the true alveoli and alveolar ducts appear. Later the lobules grow at the expense of the fatty tissue, and the lactiferous gland assumes its characteristic form. In this proliferative phase in the development of the gland the hormones of the ovaries play an important role.

The milk production capacity is of a hereditary character as regards the amount

and butterfat content of the milk. The lactation period and its duration also depend on hereditary factors, but the manifestation of these characters is also influenced by feeding, external and internal physiological conditions, age, selection, etc. The state of health is also a decisive factor, because diseases directly or indirectly decrease the volume of milk production. High temperatures or thermoregulation disorders may lead to reduced production.

The dry period: at the end of the lactation period the reserves of the organism are practically exhausted. Preparation for the next period must therefore begin in the middle of pregnancy.

All the particulars of the histological changes taking place during this period in the lactiferous gland are not yet known. It is known, however, that active production stops within a short time, and the smallest ducts suffer involution.

At the end of his paper the author gives a brief summary and draws several conclusions.

A. J. Smith: Algunos efectos de las altas temperaturas ambientales sobre productividad de las gallinas (Effect of high environmental temperatures on productivity in hens), (1976, *3*, 8).

The author discusses the subject given in the title using 56 literary sources. The work is divided into three main sections: 1. feed consumption by layers, 2. metabolic heat production of layers, 3. effect of environmental temperature on the balance of energetics and egg production.

The productivity of layers is influenced by many factors: the denotype, feeding, daylength, state of health and climate. Under intensive conditions the direct and indirect effects of the environmental temperature influence not only the number of eggs produced in a laying period but the quality of the eggs too.

The feed consumption of layers: the author evaluates the method of feeding "*ad libitum*" widely used in practice, with reference to numerous literary data. It was generally believed in the past that hens stopped eating when they had consumed enough feed to maintain their body weight.

Feed consumption is controlled by physiological mechanisms in which the hypothalamus has the leading role, primarily through heat regulation. Correlations between heat regulation and feed consumption were studied by Brobeck, who thought that if a larger quantity of feed was consumed the body and blood temperatures would increase and then, in consequence of a reaction by the hypothalamus, feed consumption would decrease. This theory explains why animals and birds consume less feed when the temperature is high than when it is low. Others think that the "glucoreceptors" of the nervous system have a role in the controlling mechanism. The author also sets forth further hypotheses and arrives at the conclusion that feed consumption is under a polyfactorial influence.

Effect of environmental temperature on appetite: the digestion decreases when the temperature of the surroundings rises. Several authors have demonstrated that the appetite decreases when the temperatures exceeds 21°C. Appetite decreases by 1.5% with every degree from 21 to 30°C, and by 4.6% per degree between 32 and 38°C. The author confirms his statement on the effect of environmental temperature on the metabolism by exact data presented in tabular form.

Among other factors influencing the digestion the author mentions the protein content of the feed as one of the most important factors. He publishes literary data to show that an additional amount of protein increased egg production. Data are also presented on the role of lysine and methionine.

Metabolic heat production of layers: the author examines the correlation between metabolic heat and egg production on the basis of numerous literary references. The basic temperature of layers was 86 cal/kg compared to 73 cal/kg in other hens.

Effect of environmental temperature on the balance of energy and egg laying: the uptake of feed decreases exponentially with the rise in the environmental temperature, and the egg production decreases proportionately as is demonstrated by citing a number of authors. The authors give different temperatures (13—15—18°C) as optima for egg

laying. Warm climates render it possible to reduce the amount of feed consumed by the hens, though there must naturally be a simultaneous increase in the protein, mineral and vitamin content of the feed. In the temperate zone the layers require more feed to attain maximum egg production. In the tropics the environmental temperature must be optimised.

Egg production is also influenced by genetic differences. In the future it will perhaps be possible to select lines capable of high egg production even in environments with high temperatures.

Ciro Pérez Troncoso: Las celulas immunocompetentes (Immune competent cells), (1976, *3*, 9—10).

The author gives a comprehensive report based on 76 literary sources on immune competent cells which play a part in the immune response.

The mechanism of immunity is discussed from two basic points of view: 1. The barrier to immunological protection, 2. Local immunity.

Local immunity. The author cites Besredka, who was the first to establish the existence of a local immune system. He used enterobacteria in his experiments, where antibodies appeared in the digestive tract and its walls without being demonstrable in the blood. A local immune response was again obtained when a culture of Bacillus anthracis was inoculated under the skin in rabbit.

The author mentions Litvinenko's work as something of great interest: agglutinines were isolated from the vaginal mycoderm of cows infected with brucellosis, while agglutination of the blood serum gave a negative result.

He cites many authors from the thirties and forties who applied various vaccines to study the immune response of the organism and the development of local immunity and found that a repeated introduction of antigen led to the "generalization" of the immunity. On the basis of the literary data the author is of the opinion that local immunity is directly connected with the immune competent cells of the individual organs, which are able to synthesize specific globulins "*in situ*". The respiratory organs, the uterus, the vagina and the lactiferous glands are able to form specific antibodies, which may migrate to other organs. A local immunity against mastitis can be brought by stimulating the immune competent cells with mitogenic substances.

The author cites several authors, including Toppley—Wilson, who produced local immunity in the nasal mycoderm of rabbits with antigens without antibodies being demonstrable in the blood.

Plasm cells. The author cites the work of Fragraeus with additional references to the investigations of other authors and gives detailed information on the morphology, reproduction cycle and biochemical activity of plasm cells, and on their role in immunological processes.

The immune globulins of the plasm cells are discussed, giving particulars about their sedimentation constants and molecular structure. With respect to the latter, the author shows that the immune globulins are built according to an identical system of 2 heavy and 2 light chains, which are polypeptides by nature. He also points out that each cell is only able to produce a single type of heavy or light chain, though in the lymphoid follicles there are cells capable of producing heavy and light chains simultaneously. According to Nossal, plasm cells capable of producing different antibodies may occur together in a particular type of tissue. In Burnet's opinion the plasm cells carry all the information necessary to identify the antigens, and in the course of the immune response clones corresponding to the antigen are proliferated.

Lysosim, properdin and betasilin, which have a bactericide effect, belong to the first barrier of the immunological defence system.

Lymphocytes and macrophages. The author summarizes the literary data, beginning with Nowell's work on the role of lymphocytes in the immune response. The sites of differentiation of the lymphocytes T and B (Thymus and Bursa, or Bursa equivalent) are described. Observations on the differences in antigen structure between the two lymphocytes are listed. The author cites works which prove beyond doubt that the T type lympho-

cytes are the mediators of cellular immunity while the B type lymphocytes are differentiated to plasm cells through a blast transformation and produce antibodies.

Trigger mechanism. The author summarizes the literature of the 1960's concerning the way in which T and B type cells evolve into memory cells.

The T type lymphocytes possess the ability to recognize the antigen, while the B type cells give a specific immune response to the antigen. In the course of the immune response a close co-operation is established between the two cell types, in which the macrophages also take part. In the immune response many mediator substances, whose functions are not fully known, also produce an effect and seem to have a joint role in the so-called trigger mechanism of the immune response.

In compiling the literature, though completeness could not be aimed at, the author showed good critical sense in selecting the most important research results of recent years in addition to fundamental works.

R. Giovanni: La cria del ternero con pasto. Primera parte (Rearing calves on green fodder. Part one), (1976, *3*, 9—10).

Permanent and seasonal pastures occupy an important place in the feeding system of North-West France. This feeding system is useful in milk production and, on certain areas, in meat production or in semi-intensive husbandry systems, but it plays a less important role in the rearing of young animals. In general, animals up to the age of 1 year are not grazed, so as to avoid juvenile parasitic infections. In spite of this 3—4 month old calves are sometimes put to pasture. Feeding young animals on green fodder is advantageous, since rearing in stalls is expensive.

The author keeps the economic aspects in view when reporting on how calves can be reared on green fodder with a maximum utilization of the pastures.

In his work a review is made of the relevant British and French literature from 1972 onwards. He discusses the behaviour of calves while grazing, primarily from the point of view of the length of the rumination period.

The author characterizes the development of the digestive process in calves, and relates it to the digestibility of the feed and the end product of digestion. He stresses that under similar conditions the calves utilize green fodder to the same extent as mature animals do. The calves utilize 70—80% of good quality fodder, and 80% of the cellulose consumed. Weaning with green fodder is a generally accepted method. The amount of fodder consumed by calves between 4 and 9 weeks of age is 20—70 kg, while consumption at weaning varies from 65—96 kg. This results in a 400—740 g daily live weight gain in the case of Ayrshire, Shorthorn and Aberdeen-Angus cattle. If the fodder consumption of the calves is less than this, the weaning weight will be lower, and although they later develop normally they never fully make up for their backwardness at weaning.

During the lactation period the amount of milk consumed influences the green fodder conversion. The literary data are not uniform, but it seems certain that the uptake of milk over a longer period, or an increase in the amount of milk over the same period, lead to disorders in the appetite, manifested in a decreased consumption of green fodder. It has also been observed that the volume of fodder consumption increases at a lower rate when the lactation period is protracted. The author presents synoptical tables to analyse and confirm these correlations.

The material compiled by the author concerning the trend of fodder uptake and appetite of calves reveals that the calf is not able to cover its energy requirements from weaning to the age of 5 months by consuming green fodder alone if a daily weight gain of more than 750 g is required. Detailed information on this subject is given in a table.

Data on supplementary feeding, i.e. on concentrates, maize, oats and other grain fodders, and hay, are also summarized.

The correlations between green fodder utilization per ha and weight gain are analysed on the basis of the literary data. Examples of the rotational utilization of pastures are given.

As to the health conditions of calves put out to pasture, the author shows that no

health problems usually arise; in fact, pasturing is better for the calves. Data on the temperature and other climatic factors are presented.

Of the diseases, attention is first called to the parasitoses. As regards prevention, the methods used in Great Britain are mentioned, and the importance of a periodical control is emphasized. In listing the gastrointestinal parasitoses the author presents data on the wide distribution of Ostertagia, *Trichostrongylus* and *Nematodira*. Among the parasitoses of the lungs the importance of Dichtiocaulus viviparus is emphasized.

In his work the author refers to 62 literary sources which are used appropriately and critically.

Institut Technique de l'Aviculture, Paris. Infecciones transmitidas por los huevos (Infections transmitted by eggs), (1977, *4*, 1).

The bibliographic survey presented by the Institute of Poultry Breeding Techniques, Paris, discusses information on the subject in several chapters outlining the history of the egg from laying to hatching, infections transmitted from the egg to the chicken, the possibilities of prophylaxis and methods of medicinal intervention.

In the introduction mention is made of the successful development of poultry breeding, in which an important role was played by protective measures for the disinfecting of breeding farms, vehicles, materials, incubators, etc.

Methods of control (concentration of poultry, density per m^2, etc.), the examination of the resistance of microorganisms to antibiotics, and preventive vaccinations greatly contributed to this progress.

Infections transmitted by the egg. The author describes in detail infections of maternal origin and demonstrates that seemingly healthy layers may transfer the pathogens of various diseases through their eggs. This is known as "vertical" transmission and may occur for diseases such as salmonellosis, Newcastle disease, infectious bronchitis, encephalo-myelitis, mycoplasmosis, colibacillosis and Marek's disease.

"Horizontal transmission" may be either direct or indirect; boxes used for the transportation of chickens, tools, etc. may play a part in it, and infection may be oral, rectal or aerogenic. The possibilities of vertical and horizontal transmission are demonstrated in the figures.

Infections caused by contamination of the egg-shell. These may be caused by bacteria from the hen's intestinal flora, by bacteria in the atmosphere, or in the genital tract, by microorganisms transmitted by the attendants, or in the water used for washing and cleaning, etc.

Some of the microorganisms are able to penetrate the egg-shell, e.g. salmonella, and microbes of the Arizona group and the PPLO. They are characterized by rapid penetration, so it is very important that the eggs should be disinfected within the shortest possible time after laying.

The intestinal flora. The digestive tract of the chicken is free of bacteria. Its flora begins to develop after the first uptake of feed. Topographically the distribution is such that the largest number of microbes is found in the appendix, where the number of *E. coli* may reach a value of 10^{-9}/g. Streptococci, saccharomyces and other fungi are also found.

The atmospheric flora varies. This variability is a function of the organic substances to be found in the air, and of the conditions under which the poultry are kept. Particulars are also given of factors which play a role in increasing the infecting ability of the germs found in the microflora. The data are illustrated with figures, and the methods used for the quantitative and qualitative microbiological analysis of the aeroflora are described. The author gives information on the culture media which are optimal for the identification of the bacteria, and presents data on the methods of sampling and checking.

Prevention of infections transferred to chickens. To prevent diseases of maternal origin hygiene must be very strict, and there must be regular checks on the genetic conditions and state of health. The medical control should include vaccinations.

For the prevention of vertical transmission the only method recognized by the author is

to break the infection cycle. He suggests several ways of preventing infection through the egg-shell, the main role being played by hygiene, cleaning, and various methods of disinfection.

Disinfection. The author describes in detail the effects of soap, surfactants, detergents and disinfectants. The standards required from the disinfectants and the conditions for their use are also discussed.

The egg-shell is porous and can be penetrated by bacteria, so the temperature of the water used for washing should be lower than 15°C. If possible the eggs should be washed immediately after they are collected. It is advisable to check the iron content of the water used for washing the eggs.

For cleaning the eggs octa-phenoxy-poly-ethoxyethanol, or various sodium salts can be used. Alkalinity promotes the penetration of the egg-shell by the disinfectant. If the eggs are dirty the solution in the washing machine should be changed every 4 hours. The action mechanism and the conditions of use of chlorinated substances and quaternary ammonium salts are also discussed.

It is difficult to suggest a general rule for treating the infections transferred to chickens as they are caused by various microorganisms under different conditions. Usually antibiotics are used. These are particularly advantageous in the case of salmonellosis. In Holland, for example, tylan is used to prevent mycoplasmosis. Methods of inoculating eggs, or immersing eggs in solutions containing drugs are also employed. The author gives directives concerning the optimum execution of these methods.

S. TUBOLY

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AUCTORES

ABO-ELGHAR M. R.
Department of Plant Protection,
Faculty of Agriculture,
Tanta University,
Shebin El-Kom,
Egypt

ALMÁSI E.
KE Élelmiszertechnológiai és Mikrobiológiai Tanszék,
1118 Budapest,
Ménesi út 45,
Hungary

ANTAL A.
Állattenyésztési Kutatóintézet,
2053 Herceghalom
Hungary

BAINTNER K.
ÁKI Élettani Főosztály,
1024 Budapest,
Kitaibel Pál u. 4,
Hungary

BAJNÓGEL J.
Belkereskedelmi Kutatóintézet,
1051 Budapest,
Vigadó u. 6,
Hungary

BARDACH S.
MÉM Élelmiszeripari Főosztálya,
1055 Budapest,
Kossuth Lajos tér 11,
Hungary

BENDE P.
Szabadegyházai Szeszipari Vállalat,
2432 Szabadegyháza,
Hungary

BODNÁR M.
ATE Agrárgazdaságtani Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

BOGDÁN E.
Állattenyésztési Kutatóintézet
2053 Herceghalom,
Hungary

BOGNÁR S.
KE Növényvédelmi Tanszék,
1118 Budapest,
Ménesi út 44,
Hungary

BOLGÁR P.
Szeszipari Vállalatok Trösztje,
1089 Budapest,
Diószeghy S. u. 8,
Hungary

CHANDRA S.
Division of Genetics,
Indian Agricultural Research Institute,
New Delhi-110012,
India

FARAG A. I.
Department of Plant Protection,
Faculty of Agriculture,
Tanta University,
Shebin El-Kom,
Egypt

FARRAG G.
Department of Plant Pathology and Agricultural Botany,
Faculty of Agriculture,
Ain-Shams University,
Shobra El-Kheima, Cairo,
Egypt

FAZEKAS S.

SOTE II. sz. Kémiai-Biokémiai Intézet,
1088 Budapest,
Puskin u. 9,
Hungary

FÜREDI J.

ATE Növénynemesítéstani Tanszék,
2103 Gödöllő,
Hungary

GABR A. I.

Department of Plant Pathology and Agricultural Botany,
Faculty of Agriculture,
Ain-Shams University,
Shobra El-Kheima, Cairo,
Egypt

GALLÓ GY.

SzSzV. Pécsi Keményítőgyára,
7601 Pécs,
Bolgár Néphadsereg u. 47,
Hungary

GERGELY I.

Gyümölcs- és Dísznövénytermesztési Kutatóintézet,
1223 Budapest,
Park u. 2,
Hungary

GYŐRI D.

KATE Talajtani Tanszék,
8360 Keszthely,
Deák Ferenc u. 16,
Hungary

GYŐRVÁRI I.

ATE Agrárgazdaságtani Tanszék,
2103 Gödöllő,
Hungary

HELMECZI B.

ATE Talajtani és Mikrobiológiai Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

HOLLÓ J.

MTA Központi Kémiai Kutatóintézete,
1025 Budapest,
Pusztaszeri út 59—67,
Hungary

HORN A.

ÁTE Állattenyésztéstani Tanszék,
1400 Budapest,
Landler J. u. 2,
Hungary

HORVÁTH GY.

Szabadegyházai Szeszipari Vállalat,
2432 Szabadegyháza,
Hungary

HORVÁTH I.

JATE Növénytani Tanszék,
6701 Szeged,
Egyetem u. 2,
Hungary

IFJÚ Z.

Gyümölcs- és Dísznövénytermesztési Kutatóintézet,
1223 Budapest,
Park u. 2,
Hungary

JÁKY A.

GYDKI Kutató Állomása,
2701 Cegléd,
Postafiók 33,
Hungary

KATONA J.

Szőlészeti és Borászati Kutatóintézet,
1022 Budapest
Herman O. u. 15,
Hungary

KÁDÁR GY.

KE Borászati Tanszék,
1118 Budapest,
Ménesi út 45,
Hungary

KÁRPÁTI L.

ATE Talajtani és Mikrobiológiai Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

KISFALVI T.

Agrárgazdasági Kutatóintézet
1355 Budapest,
Zsil u. 3—5,
Hungary

KOVÁCS A.

ELTE Növényrendszertani és Ökológiai Tanszék,
1088 Budapest,
Múzeum krt. 4/a,
Hungary

LAKATOS B.

MTA Központi Kémiai Kutatóintézete,
1525 Budapest,
Pusztaszeri út 59—67,
Hungary

LÁSZTITY R.

BME Biokémiai és Élelmiszertechnológiai Tanszék,
1111 Budapest,
Műegyetem rkp. 3,
Hungary

LŐRINCZ J.

GATE Földműveléstani és Növénytermesztési Tanszék,
2103 Gödöllő,
Hungary

MAJER J.

GATE Mezőgazdasági Kémiai Tanszék,
2103 Gödöllő,
Hungary

MAKHIJA O. P.

Division of Genetics,
Indian Agricultural Research Institute,
New Delhi-110012,
India

MÁDY G.

MTA Központi Kémiai Kutatóintézete,
1525 Budapest,
Pusztaszeri út 59—67,
Hungary

MÁTZ G.

KATE Talajtani Tanszék,
8360 Keszthely,
Deák Ferenc u. 16,
Hungary

MEISEL J.

MTA Központi Kémiai Kutatóintézete,
1525 Budapest,
Pusztaszeri út 59—67,
Hungary

NAGY J.

DATE Matematika-Fizika Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

NAGYPATAKI I.

Szabadegyházai Szeszipari Vállalat,
2432 Szabadegyháza,
Hungary

NÉMETH S.

Öntözési Kutatóintézet,
5540 Szarvas,
Szabadság u. 2,
Hungary

NYÉKI J.

KE Gyümölcstermesztési Tanszék,
1113 Budapest,
Villányi út 35—43,
Hungary

ÖRÖSI PÁL Z.

Kisállattenyésztési Kutatóintézet,
Méhészet,
2101 Gödöllő,
Hungary

PAIS I.

KE Kémiai Tanszék,
1114 Budapest,
Villányi út 29—31,
Hungary

PASCHKE H.

Állattenyésztési Kutatóintézet,
2053 Herceghalom
Hungary

PÁL A.

DATE Állattenyésztéstani Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

PÁL GY.

Acta Agron. Hung. Szerkesztősége,
2462 Martonvásár,
Hungary

PÁSZTOR K.
ATE Növénytermesztéstani Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

PECZNIK J.
ATE Mezőgazdasági Kémiai Tanszék,
2103 Gödöllő,
Hungary

PÉNZES L.
SOTE Gerontológiai Kutató Csoportja,
1085 Budapest,
Somogyi Béla u. 33,
Hungary

POZSÁR B. I.
MTA Izotóp Intézete,
1525 Budapest,
Konkoly Thege út,
Hungary

RAAFAT A.
Department of Plant Pathology and
Agricultural Botany,
Faculty of Agriculture,
Ain-Shams University,
Shobra El-Kheima, Cairo,
Egypt

RADWAN H. S. A.
Department of Plant Protection,
Faculty of Agriculture,
Tanta University,
Shebin El-Kom,
Egypt

RAJKI, S.
MTA Mezőgazdasági Kutatóintézete,
2462 Martonvásár,
Hungary

RIGÓ J.
Országos Dietetikai Intézet,
1085 Budapest,
Makarenkó u. 24.
Hungary

ROMÁNY P.
Mezőgazdasági és Élelmezésügyi
Minisztérium,
1055 Budapest,
Kossuth Lajos tér 11,
Hungary

RUFF J.
Ászári Keményítőgyár,
2881 Ászár,
Hungary

SEN S.
Department of Botanik,
Serampore College,
Serampore,
West Bengal,
India

SHMILLIÁR M.
9400 Sopron,
Lakner Kristóf u. 6,
Hungary

SÓLYOM L.
Szeszipari Kutatóintézet,
1089 Budapest,
Diószeghy S. u. 8,
Hungary

SZÁNTÓ S.
Magyar Édesipari Kutató Laboratórium,
1097 Budapest,
Vágóhíd u. 20,
Hungary

SZENDREY I.
EFE Kémiai Tanszék,
9401 Sopron,
Bajcsy-Zsilinszky u. 4,
Hungary

SZENDRŐ P.
GATE Mezőgazdasági Géptani Intézet,
2103 Gödöllő,
Hungary

SZENDRŐ Zs.
JATE Növényélettani Tanszék,
6701 Szeged,
Egyetem u. 2,
Hungary

SZÉKESSY-HERMANN V.
SOTE II. sz. Kémiai-Biokémiai Intézete,
1088 Budapest,
Puskin u. 9,
Hungary

SZUJKÓ-LACZA J.

TTM Növénytár,
1146 Budapest,
Széchenyi-sziget,
Vajdahunyadvár,
Hungary

TARJÁN R.

Országos Élelmezéstudományi Intézet,
1097 Budapest,
Gyáli út 3/a,
Hungary

THEN M.

SOTE Gyógynövény és Drogismereti Intézet,
1085 Budapest,
Üllői út 26,
Hungary

TÓTH O.

DATE Növénytermesztési Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

TUBOLY S.

ÁTE Járványtani Tanszék,
1581 Budapest,
Hungária krt. 23,
Hungary

TYIHÁK E.

Gyógynövénykutató Intézet,
2011 Budakalász,
József A. u. 68,
Hungary

VARGA M.

JATE Növényélettani Tanszék,
6701 Szeged,
Egyetem u. 2,
Hungary

VERZÁR-PETRI G.

SOTE Gyógynövény és Drogismereti Intézet,
1085 Budapest,
Üllői út 26,
Hungary

VINIS G.

KE Növényvédelmi Tanszék,
1118 Budapest,
Ménesi út 44,
Hungary

VUKOV K.

KE Konzervtechnológiai Tanszék,
1118 Budapest,
Ménesi út 45,
Hungary

ZELLER GY.

MKKE Piacszervezési Tanszékcsoport,
7601 Pécs,
Rákóczi u. 80,
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Lawickse Allee 166,
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The Netherlands.

ANNOUNCEMENT

Within the framework of the bilateral agreement between the Hungarian Academy of Sciences and the Royal Swedish Academy of Sciences, a joint workshop was organised in Hungary on June 5—9th 1978 with the title „Agricultural potentiality directed by nutritional needs".

The workshop was mainly concerned with the problem of how to double the quantity of food available for human nutrition and how to improve its quality by the year 2000.

The Hungarian Academy of Sciences invited 2 or 3 specialits from each of the European socialist countries (Bulgaria,' Czechoslovakia, German Democratic Republic, Jugoslavia, Poland, Romania, Soviet Union) to participate at the workshop, including at least one specialist in the field of food production and one in the field of human nutrition.

The Royal Swedish Academy of Sciences invited 2 or 3 specialists in the same fields from each of the Nordic countries (Denmark, Finland, Iceland, Norway).

Representatives were also present from UNESCO, FAO, WHO-, ICSU and the UN University.

The organising committee, made up of Swedish and Hungarian specialists, proposed the following subjects for discussion at the workshop:

1) Human nutritional needs with special reference to balance between protein, carbohydrates, fat and vitamins at different levels of food supply.

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PURIFICATION AND PROPERTIES OF A PHOSPHODIESTERASE AND NUCLEOTIDE PYROPHOSPHATASE FROM ROOT CALLUS TISSUES OF VINE SHOOTS

By

P. KOZMA, J. UDVARDY, M. K. SZABÓ, D. POLYÁK

HORTICULTURAL UNIVERSITY, INSTITUTE OF VITICULTURE, BUDAPEST; BIOLOGICAL RESEARCH CENTRE OF THE HUNGARIAN ACADEMY OF SCIENCES, INSTITUTE OF PLANT PHYSIOLOGY, SZEGED

Root callus tissues of vine shoots (Italian riesling) were shown to contain acidic phosphomonoesterase, phosphodiesterase and nucleotide pyrophosphatase activities. The latter two enzymes, with pH optima of 5.5 and 5.5—7.5, were partially purified (about 220- and 25-fold) by ammonium sulphate precipitation and chromatography on a DEAE-cellulose column. The acidic phosphodiesterase resembled phosphodiesterase II from animal and plant tissues in a number of general properties, including its pH optimum and its non-sensitivity to metal chelating-agent (EDTA). Bivalent ions such as Mg^{++}, Zn^{++} and Ca^{++} slightly inhibited the enzyme activity. Native DNA and high molecular weight RNA were resistant to the enzyme, while single stranded DNA was attacked; however, low molecular weight RNA and RNA-core were rapidly split. The enzyme hydrolysed poly (A), poly (C) and poly (U) at about the same rates, whereas poly (G) was resistant. The enzyme action on heat-denatured DNA was found to be exonucleolytic. The acidic phosphodiesterase exhibited the highest activity against p-nitrophenyl thymidine-3-phosphate. Other synthetic substrates, such as bis-p-nitrophenyl phosphate and p-nitrophenyl thymidine-5-phosphate were also hydrolysed. Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) natural substrates, were attacked at lower rates, while nicotinamide adenine dinucleotide (NAD) and flavine adenine dinucleotide (FAD) were slightly hydrolysed. The nucleotide pyrophosphatase resembled the enzymes purified from potato tuber and yeast. It was found to be non-sensitive to EDTA and did not require bivalent ions (Mg^{++}, Ca^{++}, Zn^{++}) for its activity. High molecular weight nucleic acids (native DNA, denatured DNA, RNA) and oligonucleotides (RNA-core) were not affected. The enzyme preparation has been compared on the basis of its ability to hydrolyse p-nitrophenyl thymidine-5-phosphate, p-nitrophenyl thymidine-3-phosphate, bis-p-nitrophenyl phosphate, NAD, FAD, ATP and ADP. The ratios of these activities were 1 : 0.5 : 0.4 : 1.9 : 1.6 : 0.5 : 0.3, respectively. Thus, the enzyme exhibited the highest activity towards pyrophosphate linkages in nucleotide coenzymes.

Introduction

It is known that the natural or induced ageing of leaves (UDVARDY *et al.* 1969), injuries to plant parts (WYEN *et al.* 1971a), various stress effects (LÁZÁR *et al.* 1973), pathogenic infections (WYEN *et al.* 1971b) and hormone treatments (UDVARDY—FARKAS 1972, WYEN *et al.* 1972) cause an increase in the activity (level) of nucleases.

Our experiments are aimed at finding characteristics at the enzyme protein level, the changes in which may provide a basis for determining the course and extent of dormancy in vine shoots. With this in view changes in the

activity of two nuclease type enzymes, phosphomonoesterase and phosphodiesterase, were studied. The evaluation of the obtained data will be published in a separate paper. The subject of the present paper is the isolation and enzymological characterization of activities which split phosphomonoester and phosphodiester bonds in various phases of dormancy.

Material and Method

In Vitis vinifera (cv. Italian riesling) shoots stored in the usual way callus formation was induced in March. Parts of internodes, 10—12 cm long, were placed in a sterilized plastic box between wet filter-paper layers. Callus formation on the shoot and root poles took two weeks in a thermostat at 35°C. The amorphous mass of cells was removed from the cutting surface with a scalpel, then weigted and washed in tap water for ten minutes. Until further use the material was kept in cold-storage (at —23°C).

The determination of phosphomonoesterase and phosphodiesterase was performed according to UDVARDY *et al.* (1969, 1970). When organic phosphorus compounds were used as substrates the method of KOLE *et al.* (1976) was followed, while the enzymatically released inorganic phosphate was measured using the method of CHEN *et al.* (1956). The protein content was determined after LOWRY *et al.* (1951). In judging whether enzyme cleavage was of the exo- or endonucleolytic type the method of BIRNBOIM (1966) was used. The base-specificity of acidic diesterase was determined by using synthetic nucleic acid homopolimers after CORDIS *et al.* (1975).

In the partial purification of the enzymes the following course was taken. All manipulations were carried out in a cold room (+3—4°C) or in cooled vessels.

1. Crude extract: 150 g callus formed on the root pole was homogenized in 0.05 M Tris-HCl buffer (pH 7.5) containing 0.1% ascorbic acid. The suspension was centrifuged in a Sorvall RC-5 refrigerated centrifuge (at 8,000 x g for 20 minutes, then at 40,000 x g for 30 minutes).

2. $(NH_4)_2SO_4$-saturation: Ammonium sulphate was gradually added to the crude extract, with stirring, up to 80% saturation. The protein precipitate was centrifuged (35,000 x g, 30 minutes), then dissolved in 50 ml 0.01 M Tris-HCl buffer (pH 7.5). Then it was dialysed overnight in a Visking tube against 5 litres of the above buffer.

3. DEAE-cellulose chromatography: A 2 cm × 30 cm column was filled with DEAE-cellulose (Whatman, microgranular) and equilibrated with 1 litre of 0.01 M Tris-HCl buffer (pH 7.5). The column was loaded with dialysate and washed with a further 100 ml of the above buffer. The elution of proteins was carried out with Varigrad, using a 0.5 M linear NaCl gradient (200 ml 0.5 M NaCl in buffer → 200 ml 0.01 M Tris-HCl buffer, pH 7.5). The flow rate was 22—23 ml/hour and 4 ml fractions were collected. The protein content and the phosphomonoesterase and phosphodiesterase activities were determined for each fraction. The profile obtained is illustrated in Fig. 3. Fractions 48—58 and 60—70, which exhibit activity against bis-p-nitrophenylphosphate, were combined. The enzymes were characterized from aliquots of these.

Results

Table 1 shows the phosphoester-splitting activity of shoot and root callus extracts. As seen from the table, the extract is able to enzymatically hydrolyse a series of inorganic and organic compounds containing phosphate ester bonds. From this point of view the root callus appears to be more active in every case.

The pH dependency of the extract in splitting phosphomonoester and phosphodiester bonds is shown in Figs. 1 and 2. The pH dependence of the phosphomonoesterase is illustrated by a one-peak curve. The pH optimum

Table 1

Hydrolysis of various phosphoester linkages by the extract from basal and apical callus tissues of vine-shoots (Vits vinifera var. Italian riesling)

Substrate	Shoot callus			Root callus			Activity:
	Activity	Spec. activity	%	Activity	Spec. activity	%	root callus/ shoot callus
p-nitrophenyl phosphate	653.3	1814.1	100.0	3165.6	1054.1	100.0	4.85
Adenosine-5'-monophosphate	218.6	607.0	33.5	435.6	145.1	13.8	1.99
Adenosine-3'-monophosphate	218.6	607.0	33.5	602.7	200.7	19.0	2.76
Glucose-1'-phosphate	0	0	0	121.6	40.5	3.8	—
Glucose-6'-phosphate	40.2	11.6	6.2	131.7	43.8	4.2	3.28
6'-phosphogluconic acid-	42.7	118.6	6.5	197.5	65.8	6.2	4.63
Fructose-6'-phosphate	32.7	90.7	5.0	187.4	62.4	5.9	5.73
Ribulose-5'-phosphate	0	0	0	10.1	3.4	0.3	—
Ribulose-1¹,5'-diphosphate	37.7	104.6	5.8	136.8	45.5	4.3	3.63
Riboflavine-5'-phosphate	60.3	167.5	9.2	227.9	75.9	7.2	3.78
β-glycero-phosphate	98.0	272.2	15.0	187.4	62.4	5.9	1.91
p-nitrophenyl phosphate	300.5	80.6	100.0	3270.8	1172.3	100.0	10.9
Adenosine-5'-diphosphate	70.1	18.8	23.3	362.3	129.9	11.1	5.2
Adenosine-5'-triphosphate	75.1	20.1	25.0	533.4	191.2	16.3	7.1
Cytidine-5'-diphosphate	80.1	21.5	26.7	563.6	202.0	17.2	7.0
Cytidine-5'-triphosphate	10.0	2.7	3.3	437.8	156.9	13.4	43.8
Sodium pyrophosphate	220.4	59.1	73.3	613.9	220.0	18.8	2.8

Crude extract: tissues were homogenized with 0.05M Tris-HCl buffer containing 0.1% ascorbic acid (pH 7.5) at a ratio of 1 : 5, then centrifuged at 8,000 x g for 20 minutes. Activity: μg P_i hydrolysed/1g fresh weight. Specific activity: μg P_i liberated/mg protein.

is 5.1. The phosphodiesterase, however, exhibits a maximum in the neutral range as well.

The probability of this is proved by the data in Table 2. As can be seen, in the first phases of purification the phosphomonoesterase activity only appears in the acidic zone. In the case of phosphodiesterase, however, considerable activity is found both in the acidic and in the neutral zone, and their ratio hardly changes during successive manipulations (1.7, 1.4, 1.4). It is thus probable that we have here two enzymes which split the same non-specific substrate to virtually the same extent.

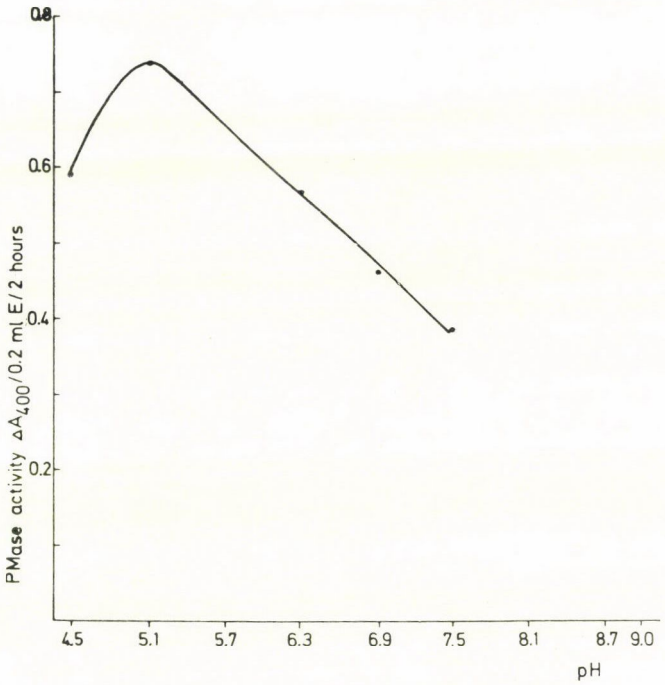

Fig. 1. pH optimum of phosphomonoesterase in the crude extract from root callus tissue of vine shoots. Enzyme extract: 8,000 x g supernatant. Enzyme was assayed with p-nitrophenyl phosphate as substrate under standard conditions except that the pH of the Tris-acetate buffer (0.1M) was varied as indicated

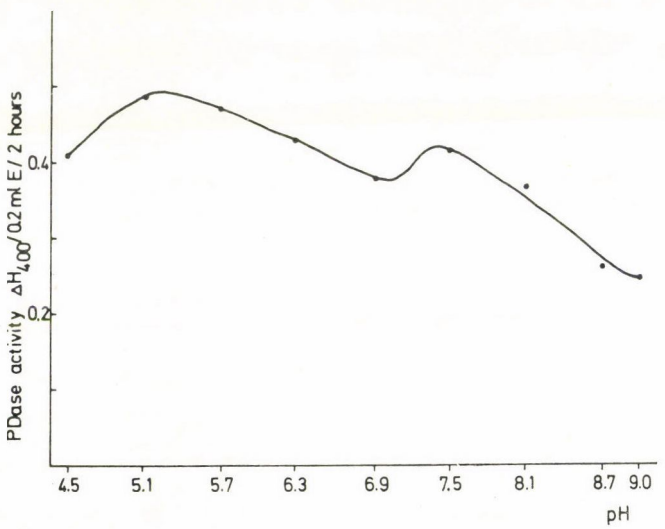

Fig. 2. pH optima of phosphodiesterases in the crude extract from root callus tissue of vine shoots. The details of the experiment were the same as those described in the legend to Fig. 1, except that bis-p-nitrophenyl phosphate was used as substrate

Table 2

Distribution of phosphomonoesterase and phosphodiesterase activities in the acidic and neutral pH ranges in various stages of purification

Purification step	pH	Phosphomonoesterase		Phosphodiesterase	
		ΔA_{400}/2 hours	pH $\frac{5.5}{7.5}$	ΔA_{400}/2 hours	pH $\frac{5.5}{7.5}$
8,000 x g supernat.	5.5	4.572	7.3	1.505	1.7
	7.5	0.625		0.885	
40,000 x g supernat.	5.5	1.725	7.5	0.450	1.4
	7.5	0.230		0.325	
$(NH_4)_2SO_4$ fraction	5.5	0.700	7.7	1.148	1.4
	7.5	0.091		0.800	

The assay system contained 100 μmoles of Tris-acetate buffer (pH 5.5 or 7.5), 5 μmoles of bis-p-nitrophenylphosphate and 0.2 ml of enzyme extract in a total volume of 1.5 ml. After incubation at 37 °C for 2 hours the reaction was stopped with 1 ml of 0.3 M NaOH and the increase in absorbance at 400 nm was measured. Substrate check was applied.

Something else can also be read from the data in Table 2. First, in the 40,000 x g supernatant only some 30—40% of the measured activities remained compared to that in the 8,000 x g supernatant. This suggests a strongly particle-bound character for the two enzymes, though the possibility that during the purification inhibitors are formed, by which the enzymes are rapidly inactivated, cannot be excluded. This is supported by the observation that in spite of the addition of 0.1% ascorbic acid to the extraction medium the homogenizate and the supernatants gradually became brown. This phenomenon is caused by the end-products of the polyphenoloxydases, which, when bound to proteins, inhibit the enzyme action.

Figure 3 shows the chromatographic separation on DEAE-cellulose of proteins purified with ammonium sulphate. When measured with a non-specific substrate, bis-p-nitrophenylphosphate, at pH 5.5, two activity peaks could be distinguished. Phosphomonoesterase activity measured similarly with a synthetic substrate (p-nitrophenylphosphate) could, however, be pointed out only in traces throughout the profile. The inactivation of the enzymes for the reasons suggest above is confirmed by the extraction percentages obtained after purification (Table 3). As seen in the table, the extraction value for phosphodiesterase in the ammonium sulphate fraction is 23% and for phosphomonoesterase 3.6%. After separation on a DEAE-cellulose column the joint value of the two phosphodiesterases is 15%, while the phosphomonoesterase

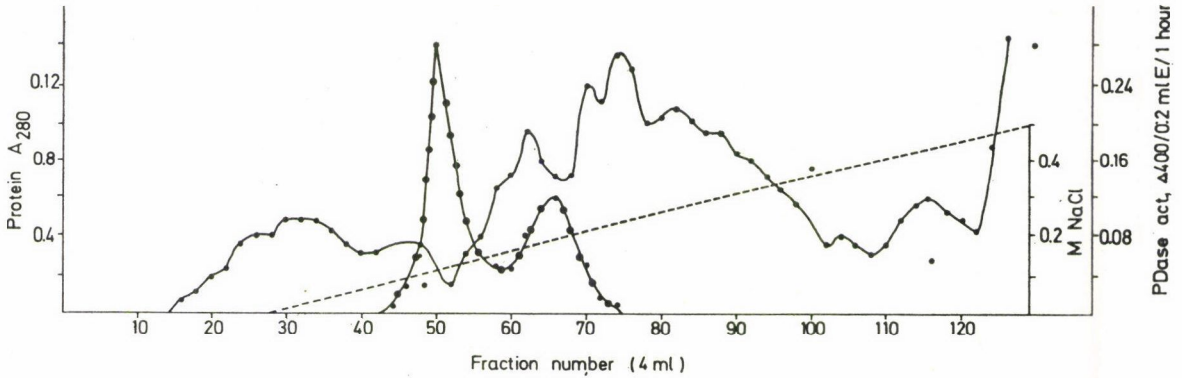

Fig. 3. Separation of bis-p-nitrophenyl phosphate-splitting activities on a DEAE-cellulose column. For details, see Material and Method. ———, protein; ·····, bis-p-NPP-splitting activities (pH 5.5); – – – –, NaCl gradient

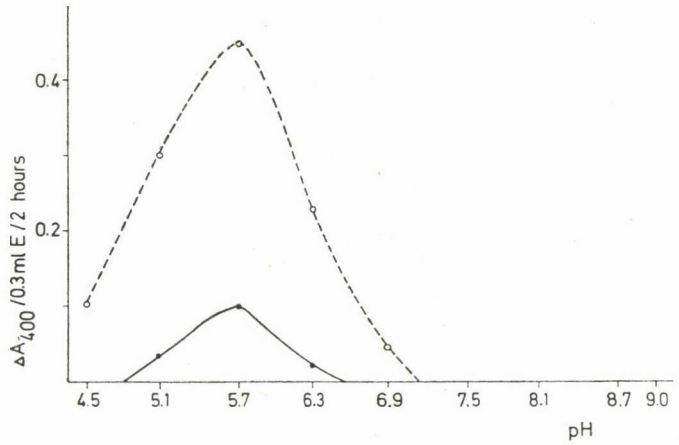

Fig. 4. pH optimum of phosphodiesterase separated by chromatography on a DEAE-cellulose column (fr. 48—58). Standard enzyme assay was carried out with Tris-acetate buffer (0.1M). – – – –, p-nitrophenyl thymidine-3-phosphate; ———, p-nitrophenyl thymidine-5-phosphate

Table 3

Purification of phosphomonoesterase and phosphodiesterase from root callus tissue of vine shoots

Fraction	Volume (ml)	Total protein (mg)	Phosphodiesterase				Phosphomonoesterase			
			Activity	Spec. activity	Purification	Yield (%)	Activity	Spec. activity	Purification	Yield (%)
Crude extract*	700	724.5	1575	2.2	1	100	6038	8.3	1	100
$(NH_4)_2SO_4$-fraction	63	31.1	362	11.6	5.3	23	220	7.1	0.8	3.6
DEAE-cellulose Fr. 48—58	34	0.376	180	479	218	11.4	Intraces	0	0	0
DEAE-cellulose Fr. 60—70	34	1.024	56	55	25	3.6	Intraces	0	0	0

* 40,000 x g supernatant made from 150 g root callus.
Standard enzyme assays were carried out. Activity: Δ^{A}_{400}/2 hours.
Specific activity: activity/mg protein.

activity is practically lost. During the separation of the activities splitting the phosphodiester bonds 218- and 25-fold purity was attained.

An attempt was subsequently made to characterize the combined fractions of the two activity maxima (DEAE fr. 48—58 and fr. 60—70) with substrates more specific than bis-p-nitrophenylphosphate. Our aim was to determine the difference in splitting ability between the phosphodiester bonds in the case of the two enzymes.

When using p-nitrophenylthymidine-3-phosphate and its isomer: p-nitrophenylthymidine-5-phosphate as substrates, the optimum pH of the first enzyme was found to be about 5.7 (Fig. 4). It split the p-nitrophenylthymidine-3-phosphate about four times better than the 5-isomer of the compound. At first sight the enzyme thus seems to be an acidic phosphodiesterase that produces a 3-phosphate end-product.

The other enzyme exhibited pH optima between pH 5.5 and 7.5 (Fig. 5). This enzyme hydrolysed p-nitrophenylthymidine-5-phosphate almost twice as efficiently as it did the 3-phosphate ester of the compound. Since, owing to its pH optimum, it cannot be placed among the alkaline phosphodiesterases (with pH optima of 8.8—9.0), further evidence is required of its proper place.

The reaction of the two enzymes to EDTA and some divalent ions is shown in Table 4. As expected, the acidic phosphodiesterase is not sensitive to

Table 4

Effect of EDTA and several bivalent ions on acidic phosphodiesterase and nucleotide pyrophosphatase isolated from root callus tissue of vine shoots

Substrate	Ions added	Concentr. (mM)	Phosphodiesterase		Nucleotide pyrophosphatase	
			Activity pH 5.5	%	Activity pH 7.5	%
p-NPT-3-phosphate	Control	0	0.500	100	0.210	100
	$MgCl_2$	2	0.325	65	0.225	107
	$ZnCl_2$	2	0.300	60	0.218	104
	$CaCl_2$	2	0.350	70	0.206	98
	EDTA	2	0.490	98	0.204	97
p-NPT-5-phosphate	Control	0	0.120	100	0.430	100
	$MgCl_2$	2	0.076	63	0.400	93
	$ZnCl_2$	2	0.078	65	0.413	96
	$CaCl_2$	2	0.074	62	0.439	102
	EDTA	2	0.116	97	0.451	105

Standard enzyme assays were carried out.
Activity: $\Delta^{A}_{400}/2$ hours.

EDTA, and its activity is mildly (30—40%) inhibited by Mg^{++}, Ca^{++} and Zn^{++} at a concentration of 0.002 M. The other enzyme was practically unaffected by the chelating agent and the divalent cations.

It is known that the phosphodiesterases (exonucleases) are able to hydrolyse certain nucleic acids. The effects of these were studied in our subsequent experiments. In this respect an essential difference was found between the two enzymes (Table 5). The acidic phosphodiesterase hydrolyses ribonucleic

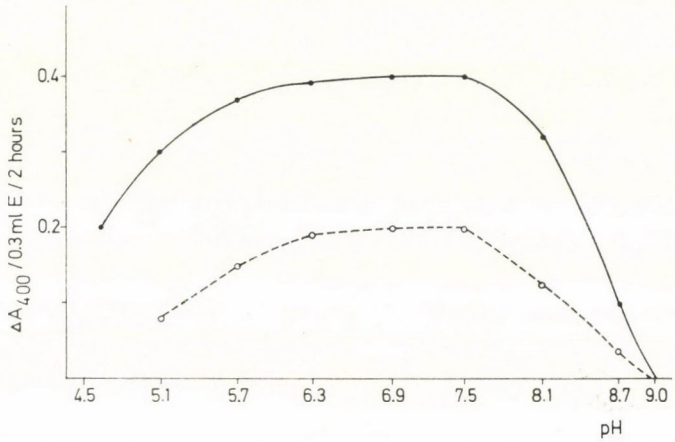

Fig. 5. Effect of pH on the activity of nucleotide pyrophosphatase separated on a DEAE-cellulose column (fr. 60—70). Standard enzyme assay was carried out with Tris-acetate buffer (0.1M). – – – –, p-nitrophenyl thymidine-3-phosphate; ———, p-nitrophenyl thymidine-5-phosphate

Table 5

Hydrolysis of various nuclei acids by acidic phosphodiesterase and nucleotide pyrophosphatase isolated from root callus tissue of vine shoots

Substrate	Conc. (mg)	Phosphodiesterase		Nucleotide pyrophosphatase	
		Activity pH 5.5	%	Activity pH 7.5	%
RNA-core*	0.5	0.400	100	0	—
RNA low mol.weight**	0.5	0.225	56.2	0	—
RNA high mol.weight***	0.5	0.010	2.5	0	—
DNA native+	0.5	0	0	0	—
DNA denatured++	0.25	0.100	25	0	—

* Ribonucleic acid-core from yeast. Sigma, Miss., U.S.A.
** Sodium nucleate from yeast. Merck, Darmstadt, Germany.
*** RNA from yeast, highly polymerized, Calbiochem, San Diego, Calif., U.S.A.
\+ From chicken blood erythrocytae, Reanal, Budapest, Hungary.
++ DNA denaturation: 2.5 mg native DNA per ml 0.1 SSC was heated to 100 °C in a water bath for 15 min. then rapidly cooled in ice.

The enzyme system contained 0.5 mg of various nucleic acids, 100 μmoles of Tris-acetate buffer (pH 5.5 or 7.5) and 0.3 ml of enzyme extract in a total volume of 0.5 ml. The incubation mixture was kept at 37°C for 2 hours. Enzyme activity is expressed as the increase in absorption at 260 nm of acid-soluble [0.3% $La(NO_3)_3$ in 2.5% trichloroacetic acid] digestion products.

acids with short nucleotide chains (low molecular weight RNA and RNA-core) to the greatest extent. To a certain degree it is capable of decomposing single stranded (denatured) DNA as well. These characters might suggest an exo-nucleolytic type of enzyme attack. The other enzyme is unable to split nucleic acids regardless of the length of their nucleotide chains.

Examinations were then carried out to determine whether there was any further difference in specificity between the two enzymes. Of the substrates employed (Table 6), p-nitrophenylthymidine-3-phosphate and bis-p-nitrophenylphosphate were best hydrolysed by the acidic phosphodiesterase. In the case of p-nitrophenylthymidine-5-phosphate, adenosine triphosphate and adenosine diphosphate the extent of hydrolysis was three or four times lower. The release of inorganic phosphorus from the 5- and 3-adenosine monophosphates was probably the result of phosphatase contamination.

The substrate specificity of the other enzyme is totally different. The extent of substrate hydrolysis, in a decreasing order, is: nicotinamide-adenine-dinucleotide, flavin-adenine-dinucleotide, p-nitrophenylthymidine-5-phosphate, bis-p-nitrophenylphosphate, adenosine diphosphate. Thus, besides splitting the di- and triphosphoester bonds to a lesser extent the enzyme is primarily specific for the pyrophosphate bonds of the nucleotides. Thus our data suggest that it is probably a nucleotide pyrophosphatase.

Table 6

Hydrolysis of various phosphoester linkages by acidic phosphodiesterase and nucleotide pyrophosphatase isolated from root callus tissue of vine shoots

Substrate	Phosphodiesterase		Nucleotide pyrophosphatase	
	Activity pH 5.5	Hydrolysis rate	Activity pH 7.5	Hydrolysis rate
p-nitrophenyl thymidine-5'-phosphate*	4.1	1	14.0	1
p-nitrophenyl thymidine-3'-phosphate*	16.5	4	6.8	0.5
bis-p-nitrophenylphosphate**	14.1	3.4	5.6	0.4
Nicotinamide adenine dinucleotide (NAD)***	2.9	0.7	27.0	1.9
Flavine adenine dinucleotide (FAD)+	0.9	0.2	21.8	1.6
Adenosine-5'-triphosphate (5'-ATP)++	5.0	1.2	7.5	0.5
Adenosine-5'-diphosphate (5'-ADP)++	5.1	1.2	4.8	0.3
Adenosine-5'-monophosphate (5'-AMP)+	0.2	0.05	0.3	0.02
Adenosine-3'-monophosphate (3'-AMP)+	0.2	0.05	0.2	0.01

* Calbiochem, San Diego, Calif., U.S.A.
** Sigma, St. Louis, Mo., U.S.A.
*** N.B.C., Cleveland, Ohio, U.S.A.
\+ P.L. Biochemicals, Milwaukee, Wisc., U.S.A.
++ Reanal, Budapest, Hungary.

The enzyme system contained 5 μmoles of various phosphoester compounds, 100 μmoles of Tris-acetate buffer (pH 5.5 or 7.7) and 0.25 ml of enzyme extract in a total volume of 1.5 ml. The incubation mixture was kept at 37 °C for 2 hours, then the sample was incubated for an additional 3 hours with 0.5 unit of alkaline phosphatase (from *Escherichia coli*, Worthington) and 33 μmoles of Tris-HCl buffer (pH 9.0). The enzymatically liberated phosphate was assayed according to the method of CHEN—TORIBARA (1956). Activity: μg P_i hydrolysed/2 hours.

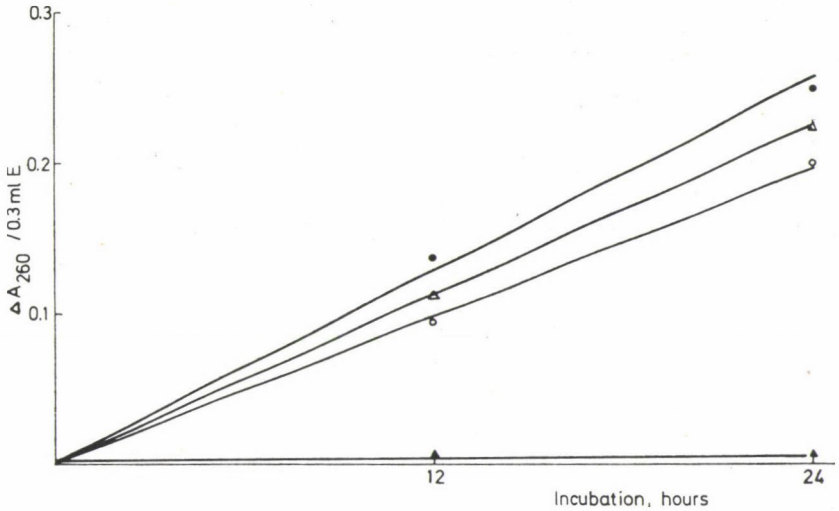

Fig. 6. Hydrolysis of synthetic polynucleotides by acidic phosphodiesterase separated on a DEAE-cellulose column (fr. 48—58). The details of the experiment were the same as those described in the legend to Table 5. ● ● ●, poly (A); △ △ △, poly (U); ○ ○ ○, poly (C); ▲ ▲ ▲, poly (G)

Attempts were also made to test the substrate specificity of the acidic enzyme, using synthetic nucleic acid homopolymers (Fig. 6). The double stranded polyguanilic acid was not split. The hydrolysis of the phosphodiester bonds of poly (A), (C) and (U), on the other hand, proved to be nearly identical. Thus, the enzyme is not specific for nucleotide base composition.

By incubating deoxyribonucleic acid with acidic phosphodiesterase and gel filtrating aliquots taken from the incubation mixture on a Sephadex G-50 column at various times experiments were carried out to determine whether the degradation was exo- or endonucleolytic. The enzyme end-product and the 3-adenosine monophosphate used as control showed the same elution profiles. Thus, the enzyme is probably an exonuclease, which splits the oligonucleotides and the synthetic substrates at the 5-hydroxyl end and produces mostly 3-monophosphate end-products.

Discussion

On studying the hydrolysis of p-nitrophenylthymidine-5-phosphate in crude extracts of pea and wheat seedlings RAZZEL (1966) distinguished two activities. Substrate hydrolysis measured at pH 9 was stimulated by Mg^{++}ion and inhibited by EDTA. The enzyme found in vine root callus can be regarded as identical with the alkaline phosphodiesterase isolated and characterized from carrot (HARVEY *et al.* 1967) and oat leaf (UDVARDY *et al.* 1970).

The other activity measured in the neutral zone was not affected by either Mg^{++} or EDTA. This enzyme was considered by RAZZEL (1966) to be nucleotide pyrophosphatase.

In vine-root callus extract, on the basis of bis-p-nitrophenylphosphate hydrolysis, an enzyme active in the neutral zone can also be found (Fig. 2). Its presence in the first phases of purification together with the so-called PD-ase II, an acidic phosphodiesterase already known and characterized, was also confirmed by the distribution of activities measured in the acidic and neutral zones (Table 2). The two activities can be separated on a DEAE-cellulose column (Fig. 3). The first enzyme peak was eluted from the column at a 0.12 M concentration of sodium chloride. This agrees with the DEAE-elution value for acidic phosphodiesterase isolated from the exudate of pumpkin (HEYSER *et al.* 1974). The elution value of the enzyme active in the neutral zone was found at 0.2 M NaCl concentration.

It is a surprising fact that in fraction obtained after chromatography on a DEAE-cellulose column the presence of phosphomonoesterase could be demonstrated only in traces (Table 3). In our experience (UDVARDY *et al.* 1969, 1970, WYEN *et al.* 1971) the enzyme is fairly tolerant to the multiphase purification procedure. In vine callus extracts, however, the end-products of polyphenoloxydase proved to be enzyme inhibitors. This circumstance means it will be necessary to use preventive agents more efficient than ascorbic acid in later enzyme experiments.

With the procedure used here the degree of purity attained for the two enzymes was 218- and 25-fold, respectively (Table 3). The low protein content, however, did not allow further purification.

The pH optimum of the first isolated enzyme peak was found to be acidic (5.5—5.7), like that of enzymes isolated from oat leaves (UDVARDY *et al.* 1969, 1970), and from pumpkin exudate (HEYSER *et al.* 1974). Of the p-nitrophenylthymidine-3- and 5-phosphodiester bonds the former was split more readily (Fig. 4, Table 4). At a concentration of 0.002 M the divalent cations (Mg^{++}, Ca^{++}, Zn^{++}) were slightly inhibitory. EDTA did not decrease the activity (Table 4). Unlike the phosphodiesterase isolated from barley seedlings (HOLBROOK *et al.* 1966) our enzyme did not split double stranded deoxyribonucleic acid at all, while high molecular weight ribonucleic acid was only decomposed to a very low extent, probably due to a slight ribonuclease contamination. The exonuclease character of the barley enzyme is not, however, supported by convincing evidence.

Single stranded (denatured) deoxyribonucleic acid was readily decomposed by our enzyme. Nevertheless, ribonucleic acid-core and low molecular weight ribonucleic acids consisting of short oligonucleotide chains proved to be the best natural substrates (Table 5). A similar order of decomposition was observed by HEYSER *et al.* (1974).

Besides splitting the di- and triphosphate bonds our enzyme also hydrolysed the pyrophosphate bonds of the nicotinamide-adenine-dinucleotide (NAD) and flavine-adenine-dinucleotide (FAD) coenzymes to some extent (Table 6). However, at the level of purity reached in our experiments it is impossible to decide for certain whether a single enzyme protein can be held responsible for the wide range of specificity observed.

With the application of denatured deoxyribonucleic acid the enzyme proved to be exonuclease, and when synthetic nucleic acid homopolymers were used, it turned out to have no base specificity (Fig. 6). Thus, the data lead to the conclusion that our enzyme (DEAE, fr. 48—58) is identical with the acidic phosphodiesterase (PD-ase II) widely found in animals and plants (RAZZEL 1968).

The other enzyme isolated has a wide range of pH optima (5.5—7.5). Similar values were obtained for an enzyme isolated and purified from potato tuber (KOLE *et al.* 1976). Our enzyme cannot be an alkaline phosphodiesterase (PD-ase I) as its activity was not increased by divalent cations (HARVEY *et al.* 1967, UDVARDY *et al.* 1970), and it did not prove sensitive to EDTA (Table 4).

The enzyme hydrolysed p-nitrophenylthymidine-5-phosphate more readily than the 3-isomer (Fig. 5). A characteristic feature of the enzyme is that it does not hydrolyse nucleic acids (Table 5), and this also applies to the oligonucleotides (RNA-core, low molecular weight RNA). Thus, in the original sense of the word it cannot be a phosphodiesterase.

Although it split di- and tri-phosphate bonds to a certain extent, the enzyme was most efficient in hydrolysing the pyrophosphate bonds of nucleotide coenzymes (NAD, FAD) (Table 6). For the substrates employed, the following rates of decomposition were observed: bis-p-nitrophenylphosphate : p-nitrophenylthymidine-5-phosphate : p-nitrophenylthymidine-3-phosphate (1.0 : 0.5 : 0.4). For the enzyme isolated from potato tuber the ratios were similar (1.0 : 0.5 : 2.1; KOLE *et al.* 1976), while for that isolated from yeast they were quite different (100 : 1 : 0; HAROZ *et al.* 1972).

On the basis of our own data and the analogues cited our enzyme can thus be considered to be a nucleotide pyrophosphatase. However, the low purity achieved does not allow far-reaching conclusions on specificity to be drawn.

In summary, phosphomonoesterase and three "phosphodiesterase type" enzymes have so far been detected in root callus extracts of vine. The characteristic features of enzymes considered to be acidic phosphodiesterase and nucleotide pyrophosphatase are described in this paper. The third enzyme, an alkaline phosphodiesterase, as well as the phosphomonoesterase will be characterized in a later publication.

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EFFECT OF BITUMEN ON PLANTS

By

V. FRENYÓ

INSTITUTE OF PLANT PHYSIOLOGY, EÖTVÖS UNIVERSITY, BUDAPEST

In an application for a patent the inventors (L. Sárosi and Á. Székely) suggest the use of bitumen solutions, emulsions, or bentonite suspensions to improve the water retension of certain soils. They inject 10 litre/m^2 of a bitumen solution into the soil at a depth of not quite 1/2 m, where it spreads as a dark layer a few mm thick and then diffuses to 8—10 cm in decreasing concentrations. This bitumen layer is designed to check the escape of water, which seems reasonable, especially in the case of sandy soils or even sandy deserts. The question is, however, what the response of plants and their roots to the bitumen treatment will be. To find the answer to this highly important question experiments were carried out on various plants under laboratory conditions. It was concluded from the experiments that the treatment of soil with bitumen as suggested by the inventors is not harmful to vegetation.

Introduction

The question of whether bitumen, or solutions and emulsions containing bitumen cause damage to plants has been discussed at length. The question has one of its sources in the fact that those working with asphalt, and workers in regular contact with tarry products, often show signs of injury. On the other hand, bitumen and asphalt are known to be attacked by many soil bacteria (FEHÉR 1954), that is, they do not inhibit the life functions of the bacteria. In fact, aromatic and paraffin hydrocarbons may, due to their nitrogen and sulphur contents, serve as food for microorganisms belonging to the genera *Vlavobacterium, Pseudomonas, Microbacterium* and *Micrococcus.*

The other source of the question may be the fact that bacteria which oxidize bitumen, paraffin, resin and kerosene display denitrifying activity too, naturally under anaerobic conditions (FEHÉR 1954). Since denitrification in the soil is a harmful phenomenon, this might be the reason why experts only indirectly concerned with the problem have raised objections to the application of bitumen in the soil. However, a loss of nitrogen in the soil can easily be made up for by the application of fertilizers containing nitrogen, and through soil cultivation techniques ensuring a granular soil structure and thereby adequate aerobiosis. Recent soil science manuals (STEFANOVITS 1975) do not even discuss such effects of bitumen.

In recently published papers dealing with the pedological and agricultural aspects of bitumen (THUM—LANGE 1971, MILCIC—MIHALIC 1972, KRONBERGER —HALBWACHS 1975, OSAFO—MILBOURN 1976) no reference to a possible phytopathological effect of bitumen is made.

Material and Method

Germination experiments were carried out on "Bánkúti 1201" winter wheat in sand previously mixed with 1% bitumen while heated in a metal tub. After it had cooled down the bituminous sand was placed in Petri dishes, wetted with tap water, then planted with wheat seeds spaced at 1 cm. As a control, wheat was germinated in the same way in pure sand. Both variants were kept in a thermostat at 22 °C for four days, then the lengths of 3 roots on each germinating seed and of the coleoptile, which is the shoot primordia, were measured. The results were averaged, and the data of the variant treated with bitumen were compared with those of the untreated control *(Experiment 1a)*.

The effect of bituminous sand on roots at a higher stage of development was then studied. For this purpose wheat grains pre-germinated for 4 days on filter paper wetted with tap water were used. Specimens with three roots were placed in wet sand and in bituminous sand similarly wetted with tap water. After a further 4 days (8 days in all) the data of these plants were also recorded *(Experiment 1b)*.

In the above experiments no solvent was present with the bitumen. Next experiments were made with bitumen dissolved in petroleum; 30% and 15% solutions were used and 15% bentonite was added to the latter, as the invention also contained bentonite. These additives were applied to the sand in ratios of 1/5 and 1/10. The medium was then wetted to full water capacity and planted with wheat seeds in the manner described above. Again wheat was planted in pure sand as a comparison *(Experiment 2a)*.

In the next stage it was again the response given by roots at a more advanced stage of development to a 30% solution of bitumen in petroleum which was studied. In this experiment wheat was grown in 500 ml Erlenmeyer flasks. After two weeks the roots of the plants formed a clearly visible thick web at the bottom of the flasks. A layer of 10 ml bituminous solution was applied to this root system. In the case of the control tap water only was added to the root system *(Experiment 2b)*.

A 10% bituminous solution was added to liquid cultures of maize and *Sinapsis alba* which were several weeks old and they were stirred from time to time to ensure intensive contact between the roots and the emulsion formed. The controls were naturally given only aqueous culture fluids. The results were followed for a week *(Experiment 3)*.

Germinating potato tubers cut in half were examined to determine whether or not a solution of bitumen in petroleum would cause injuries when brought into contact with the area of the wound. The potato tubers, with the wounded surface downwards, were buried in wet or bituminous sand layers and followed with attention for a week *(Experiment 4)*.

Wheat and maize leaves were infiltrated with bituminous solutions. When a petroleum solution was used infiltration could only be carried out by means of a vacuum. Solutions made with low surface tension benzene were able to penetrate into the intercellular space simply by capillary force. For a period of two hours the variants were kept completely covered with the solution, or in the case of the control, with tap water. After the treatment the leaves were compared, then made to float on tap water while the changes were followed for another 24 hours *(Experiment 5)*.

On a distorted model the invention was tested placing a 1 cm layer of a 30% solution of bitumen in petroleum at a depth of 10 cm under wheat plants grown in glass vessels. In the control no bitumen treatment was applied. The sand was wetted with tap water to full capacity and the development of the wheat plants was followed with attention for 1 month *(Experiment 6)*.

Results

Experiment 1a: effect of bituminous sand on the germination of wheat. The 3×10 control seedlings and the same number of bituminous variants appeared to be equally normal on the fortieth day of germination. Each seed

developed three roots; the middle one, the root proper, developed from the radicle, while the two lateral ones, the adventitious roots grew out from the mesocotyl. In fact they hardly differed from one another at this initial stage of development.

Besides the roots the coleoptile, or shoot primordia, also appeared. The sizes of these are compared in Table 1.

Table 1

Measurements of wheat seedlings on the 4th day of germination

Control, in wet sand			In wet sand, with bitumen		
Average length of a single root	Average length of the root system	Average length of the coleoptyle	Average length of a single root	Average length of the root system	Average length of the coleoptyle
mm			mm		
15.2	45.6	8.2	15.5	46.5	9.4

The data in the table testify that bitumen (with a melting point of about 60 °C) did not hinder germination or the development of the different parts at all when applied without any organic solvent. On the contrary, a slight stimulative effect was observed, but it was not our aim to prove this.

Experiment 1b: effect of bituminous sand on wheat seedlings. In this experiment a previously developed intact root system had to suffer the "stress" that might have been caused by contact with bitumen. The results are shown in Table 2.

Table 2

Measurements of 8 day old wheat seedlings

Control, in wet sand			In wet sand, with bitumen		
Average length of a single root	Average length of the root system	Average length of coleoptyle	Average length of a single root	Average length of the root system	Average length of coleoptyle
mm			mm		
46.0	171.9	49.7	48.6	168.5	47.5

There are no figures in the table to suggest any adverse effect caused by the bitumen. It may be noted, however, that the average length of the whole root system is no longer three times the average length of one root, since the wheat seedlings mostly developed five roots by the eighth day. The roots developed later are naturally shorter than the first three roots. In the bituminous culture the adventitious roots are somewhat fewer and shorter than in the control, which is why the average length of the whole root system is slightly

less than that of the control. A significant difference could not, however, be demonstrated, suggesting that the bitumen was not definitely harmful in this case either.

Experiment 2a: effect of a petroleum solution of bitumen on germination. Since the control in this experiment was the same as the one used in Experiment 1a the data will not be repeated (see the left hand side of Table 1).

Bitumen dissolved in petroleum, having diffused in the wet sand, considerably inhibited (by 50—60%) the germination of wheat, especially when it contained bentonite. In the latter case the inhibition was 95%, that is, there was only 5% germination on the fourth to fifth day.

It could be clearly seen, however, that the seeds were coated with a layer of bituminous solution which was obviously a physical obstacle to respiration. It was for this reason that the following experiment was carried out.

Experiment 2b: response of a more developed wheat root system to bitumen dissolved in petroleum. The root systems of two week old wheat plants grown in Erlenmeyer flasks tolerated the 30% petroleum solution of bitumen without any disturbance. The treated root system clearly showed that most root tips emerging from the fluid had grown an average of 5 mm in the 24 hours following the treatment. After a further 24 hours the growth was only 2 mm on average. After three days the roots showed hardly any growth, but the leaves were still growing. Neither the root system nor the leaves differed from those in the untreated control. No symptoms of toxication were found. Thus, the unfavourable result of Experiment 2a must have been the consequence of a physical, and not of a toxic effect.

Experiment 3: responses of maize and *Sinapis* plants to bitumen dissolved in petroleum. The root system of 3—5 week old test plants grown in culture fluid was not damaged by 1 week of contact with a petroleum solution of bitumen. Neither the roots, nor the leaves and shoots differed in size from the respective organs of the control plants. Changes in colour were not observed either.

Experiment 4: the effect of a petroleum solution of bitumen on germinating potato tubers. The examination of the sensitivity of potatoes did not serve only for the inclusion of further test plants in the experiment, but was also designed to examine the effect exercised directly on the wounded surface of the half-tubers.

In this variant not only did the wet sand become dark from the bituminous solution, but the latter coated the epidermis of the potatoes, which were pressed into the sand with the wounded surface downward, to a height of 1—2 cm. In spite of this no substantial difference was found between the 10 treated and the same number of untreated (control) tubers during the one-week observation. The number of shoots growing from the plumules was also the same. It is worth mentioning, however, that the tissues around the cut

surfaces of the tubers, which were in contact with the bituminous solution, became softer, which meant that the turgescence of the cells diminished slightly though definite damage could not be seen.

Experiment 5: infiltration of leaves. It is a well known fact that materials introduced into the intercellular space of the leaf may exert a more direct effect on the leaf cells than when the leaves are only floated in various solutions. It must, however, be taken into consideration that when using infiltration the air is pushed out of the intercellular space, so that the respiration of the cells may become disturbed after a certain lapse of time. This was, indeed, the case about 2 hours after the vacuum introduction of bitumen dissolved in petroleum into the leaves of wheat or maize. When the solvent was benzene instead of petroleum the leaves lost their turgescence and necrotized. Owing to the low surface tension the benzene solution penetrated into the intercellular space even without a vacuum, and caused disturbances in the metabolism of the leaf.

From this experiment it can be concluded that a badly chosen solvent may cause damage, while bitumen itself does not seem to be harmful to the plant.

Experiment 6: bituminous solution deep in the soil. The five previous experiments suggested that it was worth testing, at least in a model, whether the idea formulated by the inventors in their application for a patent would prove good in practice. The model experiment could only be carried out in the laboratory under conditions much less favourable than in an outdoor trial, because the depth of the petroleum bitumen layer in the culture flasks was much less than in the field, and there was no possibility of its seeping away either. In spite of all this the experimental wheat plants showed the same development as the control plants during 1 month of observation. Damage did not occur even when the roots had completely overgrown the layer of sand which was saturated with bitumen.

Discussion

Since on this occasion our task was different from usual, inasmuch as it was not a significant difference caused by some active agent compared to the control that had to be proved, a thorough mathematical analysis of the results can be dispensed with. The task was just the opposite: to maintain the so-called nil-hypothesis, that is to demonstrate that the bituminous solution did not cause any change in the metabolism of the plants. Thus, even if there were unavoidable differences between the untreated controls and the experimental variants treated with bituminous solutions, these differences were not significant but remained within the limits of standard deviation.

This fact is evidently shown by the numerical data of Tables 1 and 2, particularly as they mostly reveal a tendency to stimulation rather than to inhibition. Thus, if the thesis to be proved is the harmless nature of the bitumen treatment, then data which perhaps deviate from the control in a favourable direction go to confirm this statement. So there can be no objection to our not supporting it by a mathematical apparatus designed to solve more complicated problems.

There was even less need for this because the six different experimental series confirmed, from highly diverse points of view, the hypothesis that bitumen dissolved in petroleum, when properly applied, does no harm to the plants examined.

The objection may be raised that the thesis itself, i.e. the subject of the patent applied for: whether the prescribed treatment would really improve the water retension of the soil, was not examined. This objection can be rejected by emphasizing that this was not the original subject of debate; the matter in question was whether or not the recommended petroleum solution of bitumen had any poisonous effect on plants. According to the evidence of our experimental results there is no fear of any toxicosis caused by bitumen.

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DECREASE AND AUTOPHOSPHORYLATION INCREASE OF THE LABILE PHOSPHATE CONTENT IN MYOSIN

By

S. FAZEKAS, V. SZÉKESSY-HERMANN, I. ÓVÁRY

2nd INSTITUTE OF BIOCHEMISTRY, AND PSYCHIATRIC CLINIC,
SEMMELWEIS UNIV. MED. SCHOOL ,BUDAPEST

The paper deals with changes in the phosphorus content of myosin. It has been pointed out that there is a "preparative phosphate content" in purified myosin which, when followed with a standard method, is characteristic of the given muscle myosin. In the m. long. dorsi myosin described here it is about 12 mol phosphate per mol of myosin. Some of the preparative P content decreases under the influence of various effects (pH, ion milieu, actin, lipid solvents), leaving a minimum of about 4 mol of covalently bonded phosphate (2 mol of Ser-P, and 2 mol of phospholipid without lipid solvents). With a suitable phosphorylating mixture the preparative P content of myosin can be increased by an average of 5—7 mol of phosphate. So the labile P content of the m. long. dorsi myosin, taking into account the values of the reduced and phosphorylated myosin patterns, may reach the 10—12 mol level and occasionally even more. The phosphate bound under the influence of the phosphorylating mixture increases the P content in both the heavy and the light chains. Phosphorylation demands Ca^{++}, though in some cases 2—4 mol of P have been found to become bound without Ca^{++} ion. The phosphorylation of myosin due to the effect of the phosphorylating mixture can be considered as an autophosphorylation, and the myosin itself as an autophosphorylation system in which endogenous, inherent proteinkinase and phosphotransferase are present. It is perhaps the function of one of the LC's, or the joint function of all three LC's. In the course of storage the phosphorylating ability gradually weakens and finally disappears. When the incorporation of phosphate in myosin is still about half the maximum, ATP splitting still shows the ATP-ase activity characteristic of freshly prepared myosin.

Introduction

As described earlier (FAZEKAS *et al.* 1974, 1976a, 1976b) myosin contains N-phosphates of a labile nature. An account has also been given (FAZEKAS—SZÉKESSY-HERMANN 1977) of myosin being obtained with a fairly standard amount of phosphate when it is prepared with a given method. This is especially true when the myosin is obtained from a particular individual muscle. This raises the idea that if the phosphate content of myosin is the result of a given method of preparation, it certainly does not represent the in vivo phosphate content of myosin, which, according to our calculations, must be less. Some of the phosphates are in acid-labile bonds. This is the reason why the release of inorganic phosphate during storage is gradual, while under the effect of changes in the ion milieu or the pH it is suddenly released. The strength of the labile phosphate bond is sequence-dependent on the neighbouring amino acids. The development of the "preparative phosphate content" in consequence of the

adjacent amino acid effect in the course of purification is easy to imagine and explain. On this basis it can be supposed that with a suitable method of phosphorylation the decrease in the preparative phosphate content of myosin can be enhanced. The reasonableness of the idea was confirmed by the circumstances mentioned above, and moreover the effect of numerous compounds, including actin, was observed to reduce the P content of myosin, justifying us in thinking that the labile P content in myosin changes. So it is thought that the phosphorylation and dephosphorylation of myosin are probably normal, physiological, in vivo processes, at least in the skeletal muscle myosin examined here.

Material and Method

For the routine preparation of purified myosin a single m. long. dorsi muscle from 4—5 month old chinchillas was used to avoid the heterogeneity resulting from the mixing of muscles (FAZEKAS *et al.* 1974). Myosin was extracted from 100—150 g ground muscle and purified by Szent-Györgyi's method (SZENT-GYÖRGYI 1947) modified by HASSELBACH—SCHNEIDER (1951) and supplemented by ultracentrifugation and gel filtration or chromatographic separation. The extraction was only continued for 10 minutes, whereby the amount of secondary material extracted was substantially smaller, but the extraction product was also much less. The crude myosin was ultracentrifuged at 105,000 g for 2 hours. The supernatant containing the transparent myosin was gel-filtrated on a Sepharose 4B column (3.4×95 cm, 15 ml, 14—18 mg/ml myosin), or chromatographed on a DEAE-cellulose column (Whatman DE_{52}, 2.3×45 cm) using the salt gradient method. From the column only the main fraction, obtained from the DEAE-cellulose column with a solution of 10 mM $NaHCO_3$, 1 mM DTT,* 1 mM $MgCl_2$ and 0.3 M KCl, was pooled. The recovery in the case of m. long. dorsi myosin was about 90—95%. The gel-filtrated or chromatographed myosin was obtained with 11—12 mol P per mol of myosin (478,000 g). The ultracentrifuged myosin contains about 2—6.5% secondary material, and hardly less phosphate than the gel-filtrated myosin. In our studies ultracentrifuged myosin was used in many cases instead of chromatographed or gel-filtrated myosin, with satisfactory results.

The myosin was dissociated into light (LC) and heavy (HC) chains with 4.5 mol of NH_4Cl by Leger—Marotte's method (LEGER—MAROTTE 1975), or in 5 mol of guanidine-HCl in the presence of 1 mM DTT, 1 mM $MgCl_2$ and 50 mM $NaHCO_3$, adding the dissociating salts continuously by magnetic mixing in an Ehrlenmeyer flask. Finally it was diluted to 2 volumes and dialysed against 8 mM $NaHCO_3$ until the HC fraction precipitated. [Precipitation with citrate, $(NH_4)_1SO_4$, ethanol or dilution, and the use of EDTA, EGTA** and DTNB*** were avoided.] The LC fractions were further dialysed against 8 mM $NaHCO_3$, and the HC precipitates were pooled by centrifugation. The LC fractions were separated into A_1, DTNB and A_1 chains on a 2.3×30 cm DEAE-cellulose column in 2 M guanidine-HCl according to JAKES *et al.* (1976), or by developing a KCl gradient in the presence of 10 mM $NaHCO_3$ and 0.1 mM DTT. The secondary materals are eluated up to 65 mM KCl, then the LC fractions are eluated successively at 104, 135 and 200 mM. In phosphorylated myosin minor fractions are found even within the 200—300 MM KCl concentration range. The eluation of these is delayed owing to the higher P content.

The protein content was determined by Kjeldahl's nitrogen test as well as on the basis of the dry matter content and UV absorption. The optical density (A) of 1 mg/ml myosin is 0.57 at 280 nm, that of 1 mg/ml actin is 1.12 at pH 7.2 and 280 nm, and that of 1 mg/ml of the LC fraction is 0.25. In a few cases this was not satisfactory, so the dry material content and protein concentration of each sample were measured with Goa's microbiuret method (GOA 1963).

* Dithiothreitol Calbiochem.
** EGTA Koch light preparation.
*** DTNB Fluka.

The P content of myosin was determined after FISKE—SUBBAROW (1925) in the inorganic residue of samples gained after oxidation with HNO_3, but the final reduction was reached with 1% ascorbic acid (adding 1 ml for 10 ml final vol) by the method of LOWRY *et al.* (1954). The P content was calculated for the dry material, related to the mol. wt of myosin (478,000 g).

The phosphorylation of myosin was carried out in two steps. Phosphorylating mixture I contained Mg-ATP (80 mM $MgCl_2$, 10 mM ATP), while mixture II contained NaCl—$CaCl_1$ (100 mM NaCl, 0.2 mM $CaCl_1$, 5 mM $NaHCO_3$).

In general 4—5 ml (100—120 mg) myosin, containing 100 mM TRIS-HCl buffer (pH 7.6) and 0.1 mM DTT, was diluted 4—5-fold so as to reach a KCl concentration of 100—120 mM and a protein content of 7—10 mg/ml. After 5 minutes of preincubation at 30 °C, phosphorylation was initiated by the rapid, successive addition and stirring of 2 ml of solution I and 1.2 ml of solution II. In the incubation mixture the concentrations of $MgCl_1$, ATP, NaCl and $NaHCO_3$ were 6, 1.25, 25 and 2 mM, respectively, while $CaCl_2$ was present at a concentration of $2—4\times10^{-5}$M. After 30 or 60 seconds of incubation, the phosphorylation was stopped by adding 4—5 vol of icy distilled water which immediately induced the formation of myosin flakes. The precipitate was centrifuged and the supernatant was poured off. The phosphorylated myosin was suspended in a small amount of "washing solution" (20 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 1×10^{-5}M $CaCl_2$, 8 mM $NaHCO_3$), then diluted to 10—15 vols and centrifuged so as to remove the nucleotide and the free phosphate. On one occasion the phosphorylated myosin was stored overnight in the washing solution, whereby the diffusion of the superfluous nucleotide became complete. After the phosphorylated myosin had been washed and centrifuged the fifth time it was dried at 105°C, and the P content was determined in 4 aliquot (5—15 mg) parallel samples as usually as the inorganic residue.

The nucleotide content in the phosphorylated myosin and actin was determined by Asakura's perchloric acid method (ASAKURA 1961). The spectra of the supernatants were also examined and the UV absorbance was measured. From the values obtained at 259 nm the nucleotide content was calculated.

The above method seems to be disadvantageous when large quantities of myosin are used. After washing for the fifth to sixth time only 20—25% of the myosin is left for the determination of the P content. According to our observations, after the third washing neither free P nor nucleotides are to be found and further washing operations were only carried out as a precautionary measure.

Results

In an earlier work (FAZEKAS *et al.* 1976a) it was demonstrated that some of the phosphates contained in myosin are found in acid-labile bonds. This is why in the course of storage they are gradually released, though with the change of the ion milieu and H^+-ion concentration (and even during the trypsin digestion of myosin) the release of some inorganic phosphates may be sudden. The change in the stability of the phosphate bond on the effect of nearly 30 simple and combined solution systems was examined. The conclusions arrived at are shown in Table 1.

Variable conditions cause a definite decrease in the P content. The efficiency of our work is, however, based on the retention of labile phosphates. The data of the gradually decreasing P content suggested that this might be possible. The bond strength and sensitivity of N-phosphates vary due to the influence of the polar groups of the adjacent amino acid residues. If the labile phosphates originating from phosphorylation are to be observed and measured a "washing solution" is needed that will remove the free inorganic phosphate and the nucleotide, but leave the labile P content bound covalently, despite the inevitable change taking place in the conditions during the analysis and

Table 1

*The effect of different solution mixtures on the phosphorus content of myosin (mol P/478,000 g of myosin)**

Incubated	Control	In the absence Ca++ of ATP	In the presence of ATP		
			without metal ion	Ca++	Mg++
		0.2 mM	–	0.2 mM	0.2 mM
In the absence of Tris-buffer, pH less than 6, washed with icy dist. water	11.47	7.3	7.06	3.6	3.4
In the presence of Tris-buffer, washed with icy dist. water	8.64	5.4	7.98	6.4	9.21
In Tris-buffer (pH 7.8), washed with Tris-buffer	12.5	7.03	10.9	11.2	8.82
In Tris-buffer (pH 7.8), washed with diluted buffer	12.8	4.85	10.0	7.0	6.0
Frozen myosin stored at −5 °C for two days, incub. in the presence of Tris-buffer washed with diluted Tris-buffer	11.4	3.74	3.66	3.78	3.93

* All P content data are averages of three individual preparations and four parallel determinations.

structural examination of myosin. The composition of the "washing solution" described in the methodological section, when all things are considered, more or less satisfies the requirements. The effect exerted on the P content by the "washing solution", distilled water, low ion strength, actin and a range of pH in different solutions was examined and compared. The data obtained are pooled in Table 2.

The data in the table reveal that distilled water, low pH and ion concentrations and actin substantially decrease the P content of myosin. On the other hand, the P content of myosin washed three times in the "washing solution" hardly changes. Both in the control and in phosphorylated and washed myosin, nucleotides are only found in traces. In myosin pretreated with actin the nucleotide content released by perchloric acid treatment does not correspond to the starting nucleotides/mol of protein ratio referred to the actin and myosin in actomyosin, some of it having been lost in the course of washing, with only a smaller proportion (50—70%) left behind.

Table 2

Effect of "washing solution" and F-actin on the phosphate content of myosin

Treatment	P-content of myosin (mol P/mol of protein)	
Control	11.8	(10.9—12.4)
Washed with:		
washing solution	10.95	(10.6—11.7)
distilled water	6.7	(5.8— 8.1)
diluted buffer (pH 5.9)	6.6	(5.4— 8.2)
Effect of actin washed with washing solution:		
1 mol/mol myosin	8.8	(7.7—10.5)
3 mol/mol myosin	5.7	(4.7— 8.5)
4 mol/mol myosin	3.8	(3.4— 5.8)

Actin contained 3.8 g molecule P/monomer, corrected for myosin.

The data of both tables suggest that the process may also take place in reverse. In other words, the apparently stable P content of the myosin can be increased. Since the unstable phosphate was earlier found (FAZEKAS *et al.* 1976a) to form an N—P linkage in suitable amino acid residues, it is thought to be this P content which is reduced to the "preparative concentration" in the course of isolation; in other words, it is supposed that it can not only be decreased but also increased to a given higher value.

The possibility that the increase in the P content is due simply to the absorption of inorganic phosphate may render the reality of the P content of myosin questionable. This is, however, contradicted by the fact that the myosin never absorbs more than 1—2 mol of inorganic phosphate, and more than half of this can be removed with the "washing solution".

In the meantime a phosphorylating mixture was composed which increased the P content of myosin, so that it contained much more phosphate than the control even if the superfluous nucleotide phosphate was removed with icy distilled water (provided the pH was not lower than 6.3—6.4). These results are shown in Table 3.

The data of the table were not obtained with an "ideal" phosphorylating mixture, and were in fact obtained after a 5 minute incubation. In spite of this they demonstrate a decrease in the phosphate content in the control without ATP and an increase for myosin treated with phosphorylating mixture. Looking at the two extreme (decreased and increased) values of the P content in myosin the change in the labile P content is found to be substantial, some 10—12 mol. This increase in the labile P content suggests an enzymatic phosphorylation.

Table 3

Preservation and increase of P content in myosin

Incubation mixtures* Washing solution**	Control before incub.	Without ATP	Incubation mixture	
			E	E_1
	(gmol P per 478000 g myosin)***			
Precipitated and washed with icy distilled water (pH 6.3)	8.64	5.74	14.0	16.82
Precipitated and washed with washing solution (pH 8.2)	11.5	7.0	11.0	16.7
Precipitate washed with washing solution, Tris-buffer omitted	11.5	4.85	4.25	3.87

* Incubation mixtures: E: 0.2 M KCl, 105 mg myosin, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 50 mM Tris-buffer, 1.5 mM ATP; E_1: E + 10 mM NaCl.

** Washing solution: 20 mM KCl, 10 mM NaCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, Tris-buffer, pH 8.2.

*** All P content data are averages of four parallel determinations.

This is why the phosphorylation of myosin was subsequently subjected to regular examinations. The results obtained with the developed phosphorylation mixture as described in the methodological section are shown in Table 4.

The table reveals that in the m. long. dorsi of each chinchilla 4—10 mol P/mol of myosin become bound, and that this is reduced slightly but not essentially by subsequent washing (with the "washing solution").

Table 4

Increase in phosphate content of myosin as a response to phosphorylating reaction mixtures

Treatment	P content of myosin (mol P/mol protein)	
Control (before phosphorylation)	11.6	(10.4—12.4)
Phosphorylated myosin* prepared from:		
N_{227}	16.8	(15.3—17.9)
N_{229}	17.0	(16.7—18.7)
N_{230}	18.2	(16.7—19.3)
N_{231}	23.0	(21.5—24.7)
N_{233}	16.6	(15.9—17.5)
N_{234}	16.2	(15.9—17.3)
N_{235}	18.9	(16.3—19.8)

* Retained nucleotide content of myosin checked for each pattern in perchloric acid-precipitated supernatants at 259 nm. It was found to be 0.1—0.5 mol per mol myosin.

The myosin was then dissociated to heavy chain and light chain fractions. In the dissociating solutions EDTA and EGTA were replaced by 1 mM DTT, since the presence of either EDTA or EGTA in the phosphorylation mixture or dissociating solutions led to the reduction of the P content. The HC fraction was made free of lipid with a 2 : 1 mixture of chloroform and methanol applied twice and a subsequent treatment with acetone containing 4—6% distilled water; at the same time the LC fraction was exhaustively dialysed against the 8 mM $NaHCO_3$ solution, then twice against the washing solution, and separated with DEAE cellulose. The P content was then determined as shown in Table 5.

Table 5

Distribution of P content in the subunits of myosin

No.	Myosin (unphosph. control)	mol phosphate/mol of subunit***				
		HC[1]	LC[2]	A_1	DTNB	A_2
210	11.3	2.2*	0.8*			
213	11.6	4.42	1.32			
220	11.47**	4.21	1.10	0.1	1.5	0.20
222	10.95	5.05	1.35	0.15	1.7	0.35
227	12.15	4.8	1.47	0.08	1.82	0.10

1. For 420,000 g.
2. For 20,000 g of LC fractions.
* With citrate precipitation of HC.
** 2 mM EGTA in the dissociation mixture.
*** All data are averages of three chemical determinations.

It is seen that in the HC fraction 4—5 mol P/mol (for 420,000 g) and in the LC fraction 1.5—2 mol P/mol (for 20,000 g) are left behind.

The HC and LC fractions of the phosphorylated myosin were dissociated and treated in a similar way and they were examined for the distribution of the P content. The results are found in Table 6.

The table shows that the increase in the P content can be observed in both the HC and the LC fractions. The chromatographic separation and the analysis of the LC fraction reveals that the P content increased not only in Perry's 18,500 molecular weight DTNB chain but also in the A_1 and A_2 fractions. Recent investigations show that the P content of the A_2 fraction may be as much as 4 mol/mol, and the P content was successfully retained in trypsin-digested peptide fractions.

Of the results obtained by chromatography, the separation of the LC fraction of myosin for animal N_{218}, which was prepared without phosphorylation, is presented here (Fig. 1).

Table 6

Distribution of P content in the phosphorylated and dissociated subunits of myosin

No.	Myosin (control)	Phosphorylated myosin	mol phosphate/mol of subunit				
			HC	LC	individual LC		
					A_1	DTNB	A_2
213	11.6	16.5					
215	12.06	17.1					
228	13.1	17.0	9.7	2.8	0.35	1.7	0.14
228	13.1	8.1**	4.3	1.34	nd	1.56	nd
229	12.3	19.1	1.64*	1.34*	nd	nd	nd
230	9.95	17.1	4.1	1.58	0.36	1.76	0.6
231	10.0	18.2	9.1	3.1	0.7	2.4	1.15
233	11.7	17.2	9.5	3.8	1.8	2.5	4.3

* EGTA in the phosphorylation mixture.
** EGTA in the dissociation mixture.

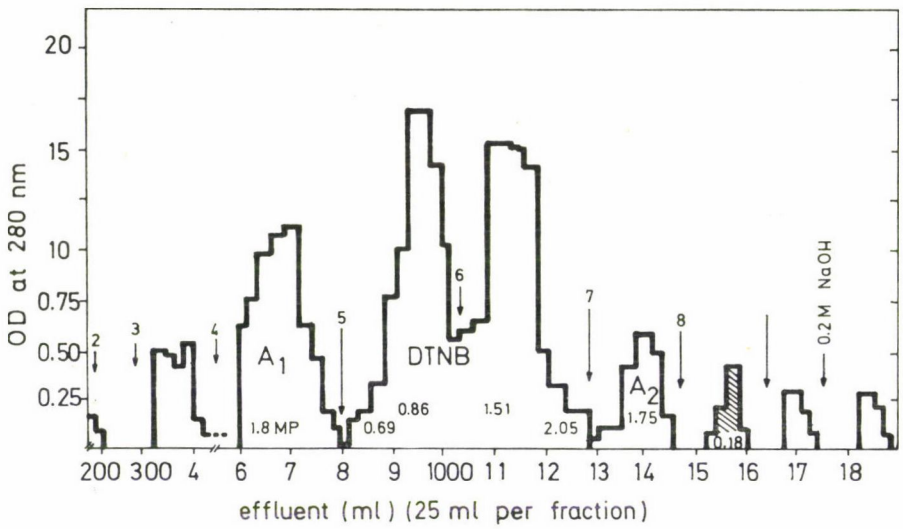

Fig. 1. Chromatography of myosin LC fractions on DEAE-cellulose column (2.3×45 cm). KCl gradients in the presence of 10 mM $NaHCO_3$, 10 mM NaCl and 0.1 mM DTT were: 1. 35 mM KCl (equilibrated solution), 2. 45 mM KCl, 3. 65 mM KCl, 4. 104 mM KCl, 5. 135 mM KCl, 6. 200 mM KCl, 7. 300 mM KCl, 8. 500 mM KCl, and 0.2 M NaOH for column regeneration

While planning and elaborating the chromatographic method it was ensured that the elution mixtures should be salt gradient buffers free of phosphate. After the different fractions seen in the chromatogram were collected

they were dialysed against a solution containing 8 mM $NaHCO_3$, 10 mM NaCl and 10^{-5} M $CaCl_2$, and the P contents were determined. The P contents of the different fractions are indicated on the chromatogram, referred uniformly to an average LC molecular weight of 20,000 g. The A_1 fraction has a considerable P content (1.8 mol P/mol of A_1), equalling that of the DTNB chain which is eluated immediately after it. Fraction A_2 was eluated according to the increasing P content and showed a P concentration of 0.91 mol.

When elaborating the method DTNB was used for dissociation into HC and LC fractions, as in the original LEGER—MAROTTE (1975) method, so the localization of the DTNB was easily recognised due to the slight yellow colour. Since the DTNB reduced the P content of the A_1 and A_2 fractions to a very low value (0.1—0.35 M) it was replaced with DTT in the dissociating solution.

A considerable part of the low P content fraction eluated by 65 mM of KCl corresponds to A_1, as the mobility and localization measured by SDS-acrylamide gel electrophoresis are identical with it. In some cases the A_2 at the end of the chromatogram is eluated in a number of minor peaks according to the increasing P content. The low molecular weight protein found at the 300 mM KCl concentration does not contain P, only in the small amount of A_2 associated with it. A smaller or larger amount of this fraction is always found in the LC fraction of non-phosphorylated myosin, but in phosphorylated myosin it cannot always be demonstrated.

The LC fractions of phosphorylated myosin behave slightly differently. It is mainly the A_2 that, owing to the presence of higher P content, shows more chromatographic peaks, the last of which may even have a 4—4.5 mol P content/mol.

The separation of LC-subunits with the NaCl gradient-2 mol guanidine-HCl method of YAKES *et al.* (1976) gives a satisfactory result when the elution buffer contains DTT, 2×10^{-5} M $CaCl_2$ and at least 10 mM KCl and 8 mM $NaHCO_3$. Its disadvantage is the prolonged dialysis against the diluted washing solution, though this hardly reduces the P contents of the individual fractions.

Discussion

A "good" phosphorylation mixture is an essential precondition for the phosphorylation of myosin. Phosphorylation demands the presence of Ca^{++}, as shown by Fig. 2. Phosphorylation starts at a Ca^{++} concentration of about 10^{-6} M and its intensity is optimum at around 10^{-5} M, but the Ca^{++} concentration must not exceed 10^{-3} M because at concentrations higher than this the P content suddenly rises, showing that the process is not enzymatic. (It is a case of inorganic P precipitation and adsorption, and there is a possibility of accumulation.)

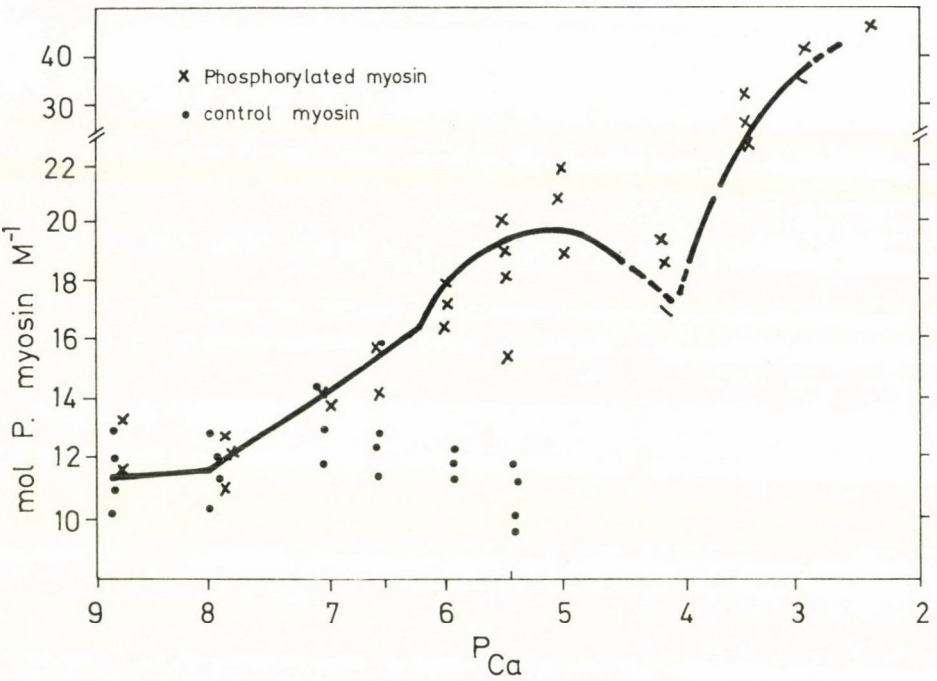

Fig. 2. Effect of Ca^{++} ion concentration on the phosphorylation of myosin. Different $CaCl_2$ concentrations were applied with the second phosphorylation mixture

As is known, NaF is a phosphatase inhibitor and its presence in the phosphorylation mixture prevents the dephosphorylation of protein. The presence of 30 mM NaF in our myosin preparations does not promote the binding of phosphate, as observed in the cardiac actomyosin preparations of REDDY—WYBORNY (1976). In other words, our preparations did not contain protein phosphatase.

It is mentioned above that one disadvantage of our experiments is that large quantities of myosin are needed to start with, as the repeated washing removes 60—80% of the myosin. However, from a different point of view they also have the advantage of providing on opportunity for checking the phosphate and lipid contents of the myosin and for determining the dry matter content. Fortunately, the two enormous m. long. dorsi muscles of rabbit offer ample possibilities for experimentation.

As shown in Table 6, improved techniques make it possible to determine the covalently bound phosphate in dissociated and chromatographically separated myosin fractions, even if the phosphate became bound in the course of phosphorylation.

Under the influence of the phosphorylation mixture the phosphate content increases both in the HC and in the LC fractions; in the former 4—5 mol, and in the latter 3—4 mol more P are measured compared to the control.

According to earlier investigations only the DTNB chain contains phosphate in the form of serine-phosphate ester (PERRIE *et al.* 1973).

Phosphate was found in all the LC fractions; in A_1 and A_2 acid-labile, and in the DTNB chain both acid-labile and alkali-labile forms occur. The results suggest that myosin should be regarded as an enzyme system which has its own autophosphorylation protein phosphokinase enzyme system. The dephosphorylation seems to be related with contraction, and the rephosphorylation with relaxation, restoration of contraction ability and the possibility of repeated triggering.

A significant decrease in the protein-bound P content of contracted muscles was observed, though not directly in myosin, by CHEESMAN—HILTON (1966), DELLUVA *et al.* (1968), who found it to be reversible on adding 3 mM Ca^{++}. They pointed out that the protein-bound radioactive phosphate can be removed by 10 mM phosphate buffer (pH 5.0) or 10 mM HCl. At that time the experimental facts were not considered to be compatible with the muscular function, so it was concluded that the "phosphate bound to an extra protein" was not the energy source for the contraction.

Later CHEESMAN—WHITEHEAD (1969) and DAVIES (1971) demonstrated with isolated myosin that the relaxed muscle was able to incorporate labelled inorganic phosphate into the myosin, and that the P content of myosin decreased on contraction.

The phosphorylation mixture used in the present case does not induce any increase in the P content of slow soleus muscle myosin. Even among the fast (white) muscle myosins only a few attained the values measured in the m. long. dorsi myosin. Therefore the phosphorylation procedure should be accepted with reservations, and should be tested before being applied to other muscle myosins.

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HISTOLOGICAL AND CHEMICAL ANALYSIS OF RED CURRANT SEEDS DEVELOPING IN VITRO

By

J. M. ZATYKÓ, I. SIMON, Cs. SZABÓ, P. GRACZA

EXPERIMENTAL STATION OF THE RESEARCH INSTITUTE FOR FRUIT TREES AND ORNAMENTALS, FERTŐD; RESEARCH INSTITUTE FOR FRUIT TREES AND ORNAMENTALS, BUDAPEST

Developing seeds dissected from 3—4 week old unripe berries of the red currant varieties F. Hosszúfürtű piros and Jonkheer van Tets were placed on Miller and Nitsch culture media. On Miller's culture medium the seeds developed well and in 3 weeks became fleshy formations suggestive of fruits. Some of them turned pink, resembling a ripe fruit. The final evaluation was made after 2 months. The cultivated seeds were then subjected to histological and chemical analyses. It was revealed that the seed coat, consisting mainly of fleshy parenchymatic tissues, thickened considerably compared to that of seeds maturing under natural conditions (Figs. 1, 2, 3), while the sugar composition of the seeds, unlike those developing and maturing under natural conditions, was similar to that of ripe red currants (Figs. 4, 5). This metamorphosis calls attention to the latent potentialities of plant organs made independent of correlation effects.

Introduction

An earlier paper reported the formation of adventitious embryos in cultivated seeds of the red currant variety F. Hosszúfürtű piros (ZATYKÓ *et al.* 1975).

Apart from the adventitious embryo formation other histological and chemical changes observed in seeds developing in vitro are not without interest either. This question is discussed in the present paper.

Material and Method

Sterile preparations were made of developing seeds from 3—4 week old unripe berries of the red currant varieties F. Hosszúfürtű piros and Jonkheer van Tets. The seeds obtained from each fruit were placed in a 100 ml flask containing Miller's (MILLER 1967) or Nitsch's (NITSCH—NITSCH 1969) culture medium. The cultures were kept for 2 months at a constant temperature of 27—28 °C and a constant intensity (about 1000 lux) of illumination, and were then subjected to histological and chemical analysis.

At the beginning and end of the experiment serial paraffin sections were prepared from the seeds. Mature currant seeds developed under natural conditions were prepared in the same way.

Besides the histological examinations seeds cultivated in vitro were also subjected to chemical analyses. Owing to the small size of the seeds only components which occur in relatively large quantities could be determined chromatographically. With this in the view sugars were the first to be taken into consideration. They were determined by the STAHL—KALTENBACH (1961) method. In the fruit of red currant fructose and glucose are the main components while saccharose is found in smaller quantities. The proportions of the sugar

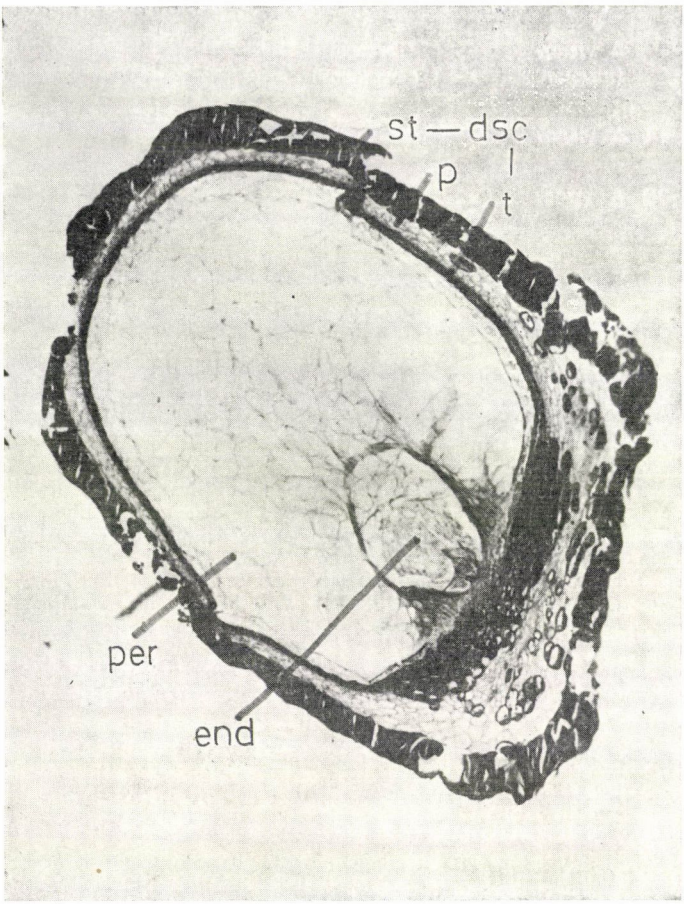

Fig. 1. Longitudinal section of immature seed of Jonkheer van Tets red currant at the time of inoculation. 200× (St = cell row containing pigment; p = parenchyma; t = thick-walled cell row; dsc = developing seed coat; per = perisperm; end = endosperm

components in a fruit are characteristic of the species, so it was hoped that valuable data would be obtained from the material examined. So that comparisons could be made from several points of view the fructose, glucose and saccharose contents were determined not only in seeds cultivated in vitro and fruits ripened in the field, but also in the petioles, roots and callus of red currant plants.

Results

In different culture media the seeds showed differences in development. On Nitsch's medium they displayed moderate growth, while on Miller's culture medium their size increased positively in 2 months: seeds of an average size of 2 mm grew to 3—5 mm. Some seeds turned pink within 2—3 weeks. The colour and fleshy texture of the formations obtained from the seeds were

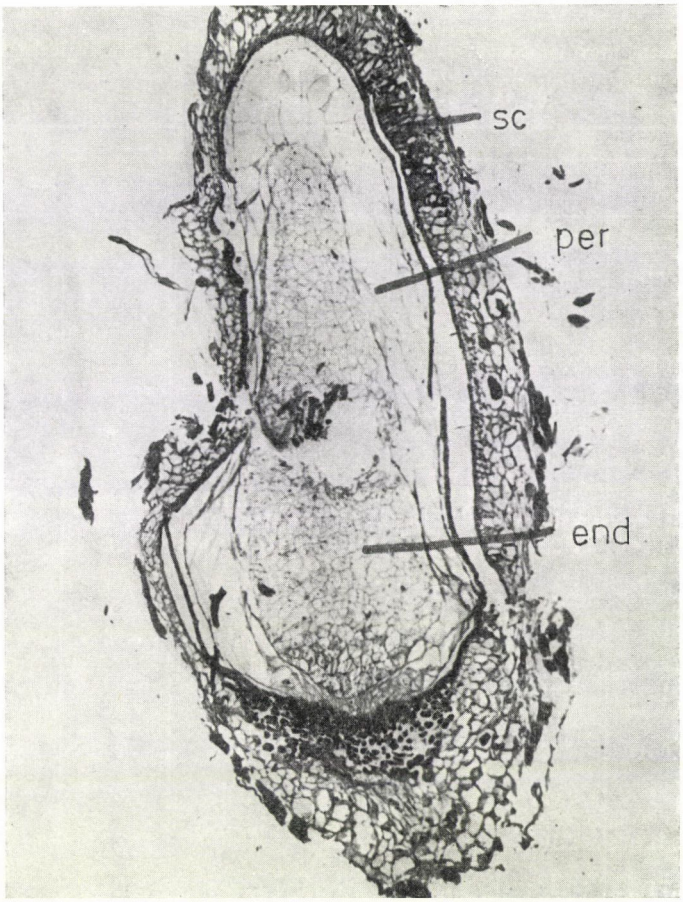

Fig. 2. Longitudinal section of Jonkheer van Tets red currant seed after 2 months of cultivation. 200× (SC = seed coat; per = perisperm; end = endosperm)

highly suggestive of the ripe fruit. Since this kind of metamorphosis was unusual, it was imperative to analyse the phenomenon with the more exact methods of histology.

In the section which fixed the initial stage (Fig. 1) the largest part of the 3—4 week old developing seed is occupied by the perisperm, formed from the nucellus. The endosperm is only visible in the form of a very small tissue zone. The young seed coat consists of a row of outer cells containing pigment, a thin-walled parenchymatic tissue zone consisting of several rows of cels and an inner cell row with somewhat thicker walls. It is clearly seen from the sections that seeds cultivated in flasks for 2 months (Fig. 2) grew compared to developing seeds freshly extracted from green berries and their seed coats grew thicker. This thickening can be attributed partly to the fact that the cells became 2 or

Fig. 3. Longitudinal section of seed from ripe Jonkheer van Tets red currant fruit grown under natural conditions. 200× (SC = seed coat; end = endosperm; emb = embryo)

2.5 times bigger and partly to the increase in the number of cell rows forming the seed coat from 4—5 to 8—9. At the same time, owing to the increase in the size of the seed, the original radial position of the outer pigment-containing layer changed and became tangential. Some cells of the underlying parenchymatic tissue zone became considerably larger than the others, and these too filled with pigment. The endosperm characteristic of the seed became considerably larger, while the perisperm was sharply reduced. The development of the endosperm was not followed by embryo organization.

In the mature seed developing under natural conditions, on the other hand (Fig. 3), the perisperm is completely used up and endosperm accumulates in its place. So the largest part of the interior of the seed is taken up, as confirmed by the littrature (HAZSLINSZKY—TAKÁCS 1960, GASSNER

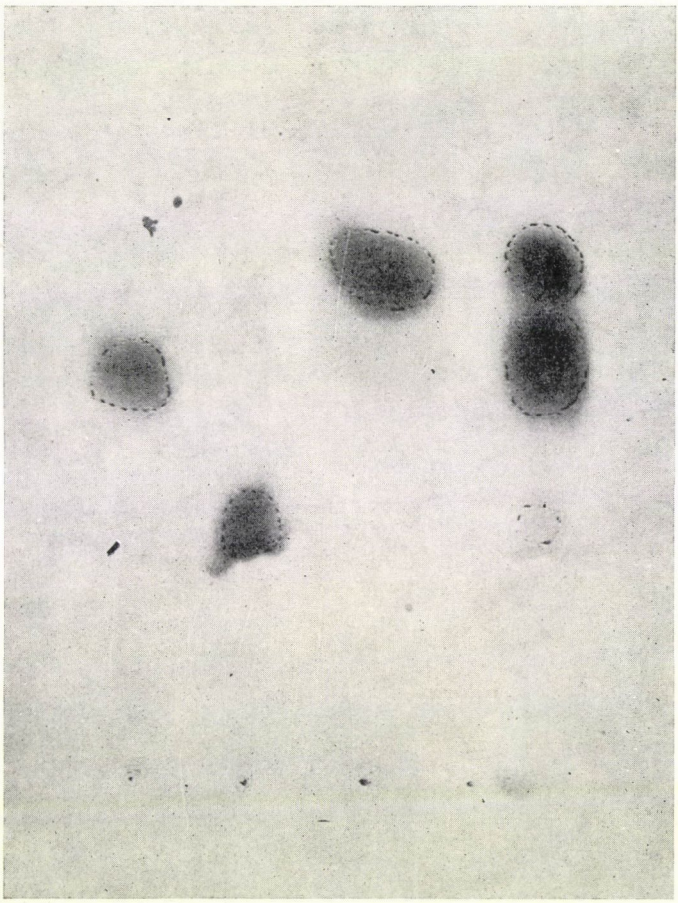

Fig. 4. Chromatogram made by the STAHL—KALTENBACH's (1961) method of the sugar components of red currant seeds cultivated in vitro. From left to right: glucose, saccharose, fructose, seed cultivated in vitro

1973), by the endosperm, in which a small embryo develops. (In the attached photo the embryo was displaced from its original position while the section was being made.) The seed coat is restricted to several rows of thick-walled cells; it is essentially thinner than the seed coat of seeds cultivated in flasks, which consists mainly of a fleshy parenchymatic tissue zone.

This basic difference between the seed coats gives a histological explanation for the fruit-like appearance of seeds developing in vitro.

Glucose, fructose and saccharose were found among the chromatographically separated sugar components of all the red currant samples (callus, root, petiole, fruit, and seed cultivated in vitro). Their proportions varied considerably with the samples, while seeds cultivated in vitro and ripe fruits showed almost complete identity in this respect (Figs 4, 5).

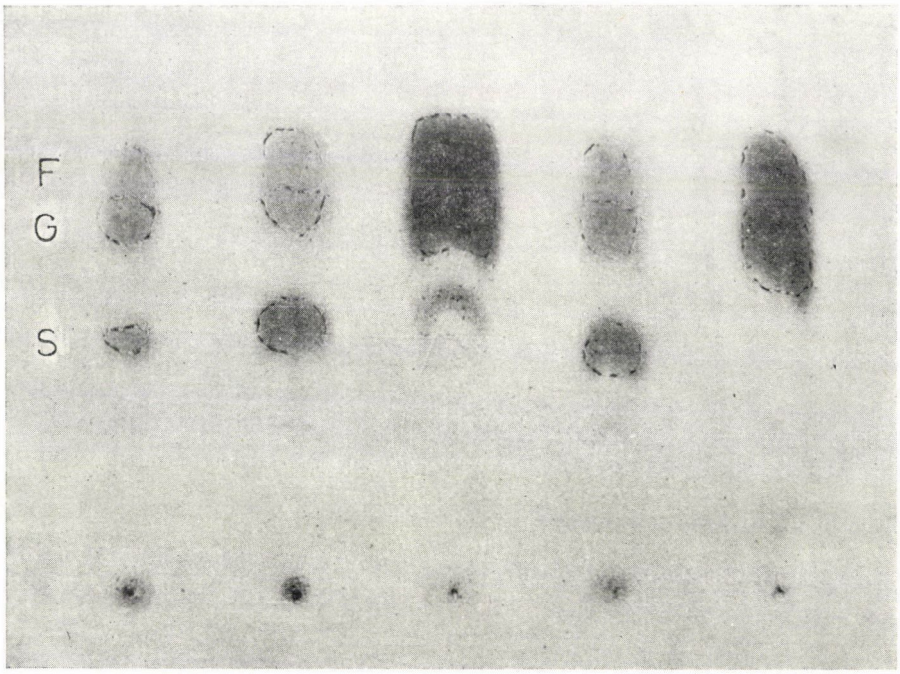

Fig. 5. Chromatographically determined sugar spectra of various organs of red currant. From left to right: root, petiole, ripe fruit, callus, seed cultivated in vitro (S = saccharose; G = glucose; F = fructose)

In the course of the quantitative analysis 7.9 ± 0.5% fructose, 6.4 ± 0.5% glucose and a hardly traceable amount of saccharose were found in both samples. The sugar composition of mature seeds grown under natural conditions was also determined, though these are not included in the chromatogram presented, owing to methodological difficulties. With the method mentioned a small amount of saccharose was found (1.2%), but neither glucose nor fructose could be detected in mature seeds.

The identical sugar composition of fruits and of seeds cultivated in vitro also underlines the fruit-like character of the latter.

Although further comparisons need to be made for a number of features between fruits and seeds cultivated in vitro, the results obtained so far already show that in developing red currant seeds removed from their natural surroundings features and tissue zones characteristic of the fruit appear in the course of in vitro cultivation.

This interesting metamorphosis, which can be followed by means of tissue cultures, calls attention to the latent potentialities of plant organs and tissues, which become active under the influence of changed environmental and nutritional conditions and produce new organizational forms.

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VARIA

WEED GROWTH IN YOUNG PEAR PLANTATIONS

In the course of investigations made on the weed plants of homogeneous fruit-tree plantations treated with various cultivation methods particular attention was paid to the effects of such environmental factors as variety, rootstock, soil, precipitation and mechanical soil cultivation on changes in weed growth. Changes in the life form and aspects, and in the taxonomic composition of the weed flora were analysed separately. The investigations outlined here are to be continued parallel with the development of the plantation.

The investigations were carried out in the pear plantation of the Kecskemét College of the University of Horticulture, at Kecskemét-Kisfái.

The soil of the area is medium heavy and slightly calcareous; the ground water level is relatively constant at about 2.5 m. The annual precipitation is 550—600 mm over the average of many years, and the annual mean temperature is 10 °C.

The six varieties in the orchard were planted as samplings in the spring of 1975. The methods of cultivation are shown in Table 1.

Table 1

Characteristic data of cultivation methods at weed survey sites (Kecskemét: Kisfái)
Date of plantation: spring 1975

Cultivation method	Spacing (m)	Trunk height (cm)	Stand density % (area covered by fruit-trees as a percentage of the total area)	
			1975	1976
A. Palmetta hedge	5×3	70	4	6
B. Oblique-branched hedge	4×2	50	6	10
C. Slender spindle	4×1.5	60	10	12
D. False-leader goblet	1.5×0.8	40	50	70
E. Orchard	0.6×0.4	15	60	85
F. Improved orchard	4.2×1	5—10	8	20

Weed control was carried out mechanically over the entire area. Weed surveys were made five times a year (Table 2) according to Balázs's method, modified by Újvárosi (ÚJVÁROSI 1973). In this surveying method the area is divided into halves, quarters, etc. Dominance (D) is expressed by six grades: e.g. grade 6 means that the species concerned covers

1/1 i.e. 32/32 parts of the quadratic area, that is, the cover is 100 percent. Transitional data between two grades can also be surveyed. The model area is 1×1 m. We worked with 35—40 squares per cultivation method and ha.

When processing the data those on the weed cover were also compared for the different methods of cultivation. The species composition of the aspects was evaluated according to

Table 2

Date of weed survey
(Kecskemét: Kisfái, 1975—1976)

	1975	1976
1.	4 May	29 April
2.	4 June	29 May
3.	21 June	25 June
4.	24 July	23 July
5.	15 September	12 September

the proportion and life form (Soó 1964, Újvárosi 1973) of the individual plant families (Soó 1964).

Besides establishing the weed associations an attempt was made to determine the ecological character of the association developed on the area in question by means of the agro-ecological index used abroad (Kovačević 1971, 1976). The requirements of

light	FO (L)
temperature	T (T)
humidity (water)	HU (F)
nitrogen	N (N) and
soil reactions	R (R)

(where the abbreviations in brackets are the German equivalents) for the individual weed species are expressed on Ellenberg's (1950, 1974) scale, complemented with relevant data given by Hungarian authors (Zólyomi *et al.* 1967, Kárpáti *et al.* 1968). The demands of species for the major ecological factors are divided into nine categories; the lowest demand is indicated by *1*, the highest one by *9*. The occasional use of *0* means that the range of demand was so wide that the high fluctuation of factors found under the conditions of Central Europe had no detrimental effect on the species (insensitive species).

Cultivation methods and weed cover. In both years the microclimate produced by the method of cultivation only affected the percentage weed cover; no difference was found in the species composition of the aspects. The smallest difference in weed cover between the two years was found for the Palmetta hedge, and the largest for the orchard (Figs 1 and 2).

The results support the opinion that the correlation between the amount of precipitation and the percentage weed cover is also influenced by the cultivation methods and cultural practices.

Changes in the extent of weed cover and the number of weed species according to the life form. As the level of mechanical soil cultivation and plant tending rises both the dominance and species number of plants in the perennial groups (H—G) show a considerable decrease, causing a relative increase in the proportion of therophytes.

With respect to the literature cited on weed coenology Timár (1957) was the first who dealt with the distribution of life forms on the South-Eastern sandy soils of the Danube—Tisza

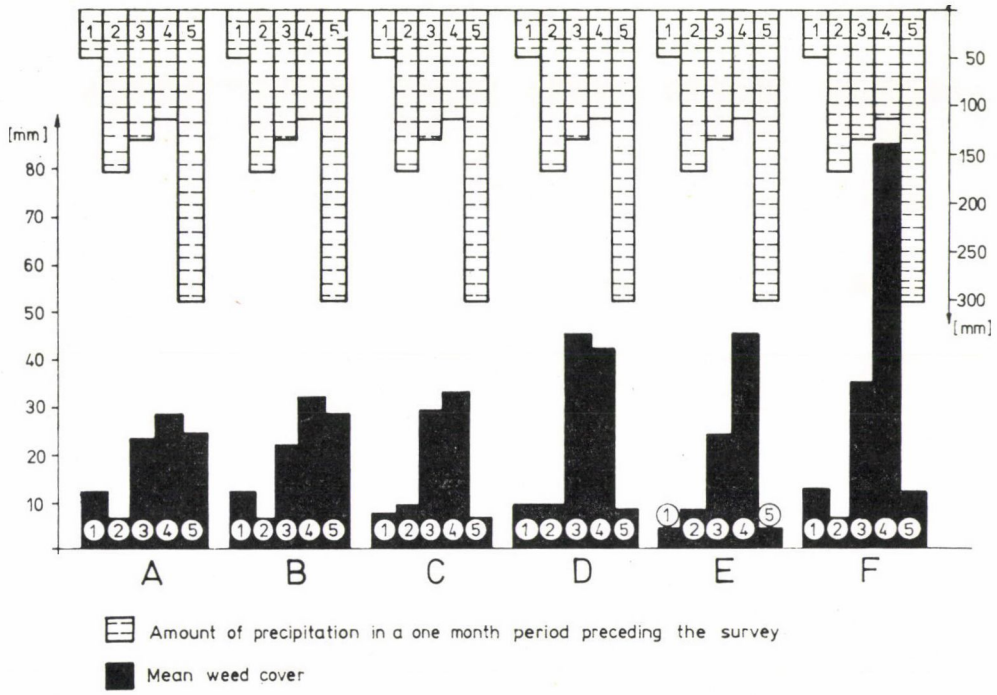

Fig. 1. Average values of percentage weed growth in young pear orchards cultivated by various methods (Kecskemét: Kisfái, 1975). 1, 2, 3, 4, 5 — dates of weed surveys (Table 2). A — Palmetta hedge, stand density 4%; B — Oblique-branched hedge, stand density 6%; C — Improved orchard, stand density 8%; D — Slender spindle, stand density 10%; E — False-leader goblet, stand density 50%; F — Orchard, stand density 60%

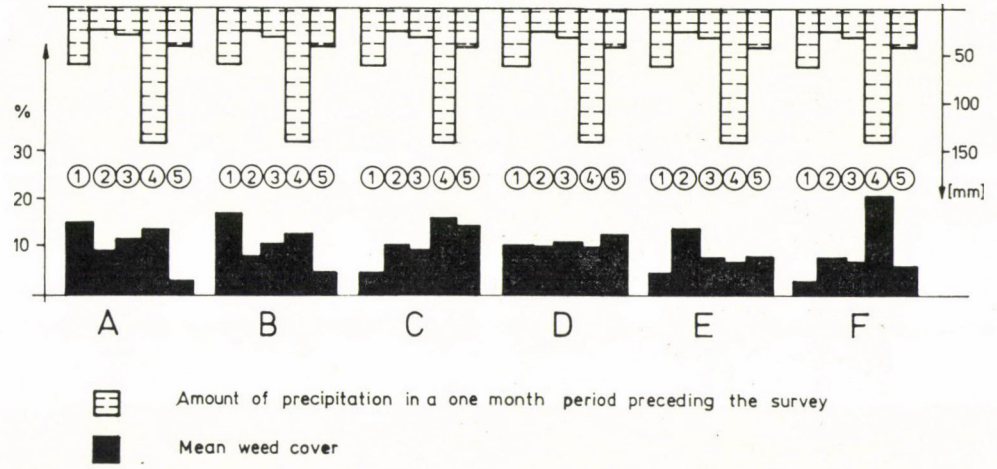

Fig. 2. Average values of percentage weed growth in young pear orchards cultivated by various methods (Kecskemét: Kisfái, 1976). 1, 2, 3, 4, 5 — dates of weed surveys (Table 2). A — Palmetta hedge, stand density 6%; B — Oblique-branched hedge, stand density 10%; C — Improved orchard, stand density 20%; D — Slender spindle, stand density 12%; E — False-leader goblet, stand density 70%; F — Orchard, stand density 85%

mid-region. According to his surveying data the proportion of therophytes in row crops was 70.2% on average, and 79.4% on drift-sand. In a weed survey made in 1961 (TERPÓ—TERPÓ M. 1962), for example, the proportion of annual species on sandy alluvial soil near the Danube was 78.2%. In recently published weed surveys the occurrence of therophytes is given as 71%. On the areas mentioned chemical weed control was not carried out.

Differences in weed growth due to the method of cultivation are expressed as a percentage of the total weed cover. Annual weed species were dominant in the young pear plantations throughout the two years with all methods of cultivation. Among the perennial weed species the geophytes took the second place (Table 4).

Table 3

Percentage distribution, according to life forms, of weed species occurring in the pear orchards examined (Kecskemét: Kisfái)

Life form	Mono-cotyledon	Dicotyledon	Total Σ	National average of the three groups
Therophyte (Th)	13	58	71	22
Hemicryptophyte (H)	1	13	14	51
Geophyte (G)	4	11	15	11
Total Σ	18	82	100	84

Table 4

Weed cover and distribution of species according to life form groups (Kecskemét: Kisfái)

Date of weed survey		Percentage weed cover				Number of species			
		Th	G	H	Total Σ	Th	G	H	Total Σ
1.	1975	8.302	1.198	0.112	9.612	13	3	2	18
	1976	8.368	*0.62*	0.128	9.116	9	3	1	13
2.	1975	*6.937*	0.48		7.417	18	2	—	20
	1976	7.116	3.09		10.256	12	4		16
3.	1975	27.722	1.94	*0.082*	29.744	19	2	1	22
	1976	7.065	2.747		9.812	10	3		13
4.	1975	*43.878*	0.483	*0.138*	44.499	9	2	1	12
	1976	12.612	0.983		13.595	6	3		9
5.	1975	12.903	1.577	0.104	14.584	11	4		15
	1976	8.525	1.566		10.091	12	4		16

Annual average cover by families. Previously neither the data obtained by coenological weed research nor those from weed surveys made immediately prior to weed control were subjected to detailed taxonomical analyses. Considering the general introduction of chemical weed control and the rapid rise in costs, it may be of great help if we determine the constancy (K) of the weed species occurring on the area and the frequency of related groups.

A phylogenetic evaluation of the major weed plant families was published in 1971. For practical purposes they were grouped as follows (TERPÓ 1975):

"A" (Widely distributed weed families of national importance):

1. *Amaranthaceae*
2. *Chenopodiaceae*
3. *Caryophyllaceae*
4. *Compositae* = *Asteraceae*
5. *Convolvulaceae*
6. *Cruciferae* = *Brassicaceae*
7. *Fabaceae* = *Papilionaceae*
8. *Gramineae* = *Poaceae*
9. *Labiatae* = *Lamiaceae*
10. *Polygonaceae*
11. *Plantaginaceae*
12. *Scrophulariaceae*

"B" (Families of locally important weed species): e.g. *Aristolochiaceae*, *Cannabinaceae*, *Equisetaceae*, *Portulacaceae*, *Ranunculaceae*, etc.

"C" (Families of sporadically occurring weed species or minor importance): *Asclepiadaceae*, *Hypericaceae*, *Primulaceae*, *Verbenaceae*, etc.

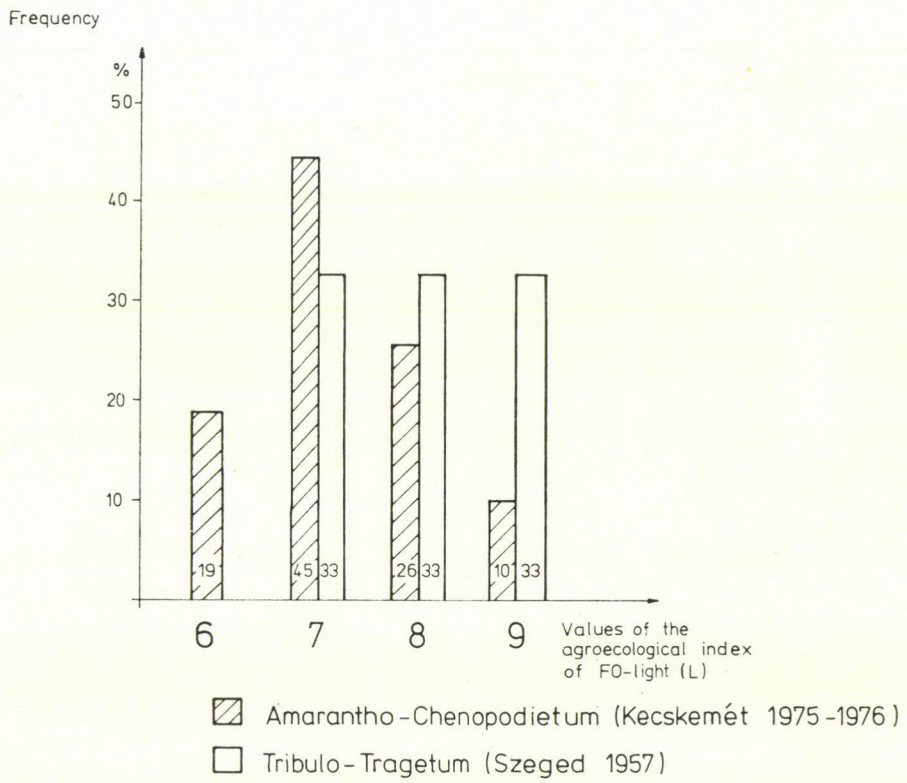

Fig. 3. Percentage frequency distribution of light indices (agroecological indices) for species of *Amarantho-Chenopodietum albi* Soó (Kecskemét: Kisfái) and *Tribulo-Tragetum* Soó et Timár (Szeged) associations

Table 5

Mean annual percentage cover of species occurring in the pear orchards (Kecskemét: Kisfái 1975—1976)

Association: *Amarantho-Chenopodietum albi* Soó;
subassociation: *portulacetosum* Bodrogk.

Life form	Number of families and species	Mean percentage cover at survey dates 1–5									
		1		2		3		4		5	
		1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
"A"	**FAMILIES OF NATIONAL IMPORTANCE**										
	Amaranthaceae 3										
Th	*Amaranthus retroflexus* L.	—	—	1.126	1.427	3.66	1.877	4.892	2.17	0.272	1.027
Th	*Amaranthus albus* L.	—	—	—	—	0.52	—	0.103	—	—	—
Th	*Amaranthus blitoides* Wats.	—	—	—	—	—	—	—	—	—	0.103
	Chenopodiaceae 2										
Th	*Chenopodium album* L.	0.917	0.177	1.078	1.885	4.83	1.247	0.622	1.508	0.458	0.283
Th	*Chenopodium hybridum* L.	0.042	—	1.204	0.078	0.053	—	—	—	—	—
	Caryophyllaceae 2										
Th	*Stellaria media* (L.) Vill.	1.078	2.758	—	0.033	—	—	—	—	0.14	0.215
H	*Melandrium album* (Mill.) Garcke	—	0.128	—	—	—	—	0.138	—	—	—
	Compositae 5										
Th	*Erigeron canadensis* L.	0.128	0.402	0.1	0.537	0.212	0.24	—	0.173	—	0.553
G	*Cirsium arvense* (L.) Scop.	0.756	0.512	0.365	4.542	0.545	1.585	0.005	0.503	0.884	0.65
Th	*Senecio vernalis* W. et K.	—	0.223	—	—	—	—	—	—	—	0.445
H	*Taraxacum officinale* F. Weber ex Wiggers	0.003	—	—	—	0.082	—	—	—	—	—
Th	*Ambrosia elatior* L.	0.003	—	—	—	—	—	—	—	—	—

Table 5 (cont.)

Life form	Number of families and species	Mean percentage cover at survey dates 1–5									
		1		2		3		4		5	
		1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
	Cruciferae 4										
Th	*Capsella bursa-pastoris* (L.) Medik.	0.02	0.10	—	—	—	—	—	—	—	—
Th	*Descurainia sophia* (L.) Webb	0.241	1.05	0.34	0.167	0.448	0.057	—	—	0.297	0.213
Th	*Raphanus raphanistrum* L.	1.86	—	0.468	0.879	1.423	0.207	—	—	0.473	—
G	*Cardaria draba* L.	0.19	0.108	0.116	0.406	0.395	0.367	0.478	0.2	0.416	0.124
	Labiatae 2										
Th	*Lamium amplexicaule* L.	1.377	3.522	0.613	0.494	1.843	0.907	0.173	—	1.037	0.302
	Lamium purpureum L.	—	—	0.138	—	0.22	—	—	—	—	—
	Gramineae 3										
Th	*Digitaria sanguinalis* (L.) Scop.	0.258	0.017	0.245	0.159	3.152	0.23	3.903	0.813	2.733	0.339
Th	*Echinochloa crus galli* (L.) P. B.	—	—	—	0.133	1.66	—	—	—	0.103	0.124
G	*Agropyron repens* P. B.	0.252	0.103	—	0.156	—	—	—	—	0.208	0.337
	Polygonaceae 2										
Th	*Bilderdykia convolvulus* L.	0.615	0.02	0.447	0.272	1.352	0.327	0.173	0.45	0.562	0.62
Th	*Polygonum aviculare* L.	0.324	—	0.447	0.347	0.38	0.207	—	—	—	—
	Convolvulaceae 1										
G	*Convolvulus arvensis* L.	—	—	—	0.987	—	0.795	—	0.28	0.173	0.379
	Fabaceae 2										
H	*Trifolium repens* L.	0.008	—	—	—	—	—	—	—	—	—
Th	*Medicago lupulina* L.	—	—	—	—	—	—	0.103	—	0.206	—

Table 5 (cont.)

Life form	Number of families and species	Mean percentage cover at survey dates 1–5									
		1		2		3		4		5	
		1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
"B"	**FAMILIES OF LOCAL IMPORTANCE**										
	Portulaceae 1										
Th	*Portulaca oleracea* L.	—	—	1.749	1.201	9.732	1.455	33.397	7.467	6.855	5.223
	Cannabiaceae 1										
Th	*Cannabis sativa* L.	1.19	—	0.045	—	0.31	—	—	—	—	—
	Malvaceae 1										
Th	*Malva neglecta* Wallr.	—	—	0.015	—	0.095	—	—	—	—	—
	Papaveraceae 1										
Th	*Papaver rhoeas* L.	—	—	0.05	—	—	—	—	—	—	—
	Solanaceae 1										
Th	*Datura stramonium* L.	—	—	0.103	—	0.103	—	—	—	—	—
"C"	**SPORADICALLY OCCURRING FAMILIES**										
	Primulaceae 1										
Th	*Anagallis arvensis* L.	—	—	—	—	0.173	—	—	—	—	—

Number of families 16; No. of species in each family 32; Mean ($\bar{x}$) = 2 species family

Table 6

Agroecological indices and frequency of occurrence of species of Amarantho-Chenopodietum albi Soó, indicating flora element characters (Kecskemét: Kisfái, 1975—1976)

Flora element	Coenosystematic groups of species	Frequency of occurrence 1975—1976	Fo	T	HU	R	N
Weed species in disturbed areas and row crops (Chenopodietea)							
Adv	*Amaranthus albus*	2	9	9	3	0	7
Adv	*Amaranthus blitoides*	1	9	7	3	0	9
Cosm	**Amaranthus retroflexus**	8	9	9	4	0	9
Adv	*Ambrosia elatior*	1	8	9	4	0	6
Cosm	*Anagallis arvensis*	1	6	6	5	0	6
Cosm	*Capsella bursa-pastoris*	2	7	0	0	0	7
Eua	*Cannabis ruderalis*	3	8	9	3	0	8
Cosm	**Chenopodium album**	10	0	0	4	0	7
Eua	*Chenopodium hybridum*	4	7	5	5	8	8
Eua	**Descurainia sophia**	8	8	6	4	0	6
Cosm	**Digitaria sanguinalis**	10	7	7	3	5	4
Cosm	*Echinochloa crus-galli*	4	6	7	5	0	8
Eua	**Lamium amplexicaule**	9	6	6	4	0	7
Eua	**Bilderdykia convolvulus**	10	7	8	0	6	6
Medit	**Portulaca oleracea**	8	7	8	4	7	7
Adv	*Senecio vernalis*	2	7	7	4	0	5
Roadside and semiruderal weeds (Sisymbrion)							
Eua	**Cardaria draba**	10	8	7	3	8	4
Eua	*Malva neglecta*	2	7	6	5	0	9
Cosm	*Datura stramonium*	2	8	7	4	0	8
Trampled weeds (Secalietea-Plantaginetea)							
Eua	*Trifolium repens* (originally a *Molinio-Arrhenatherea* species)	1	8	0	0	0	7

Tabla 6 (cont.)

Flora element	Coenosystematic groups of species	Frequency of occurrence 1975–1976	Fo	T	HU	R	N
Weed species in cereals (Secalietea)							
Eua	*Papaver rhoeas*	1	6	6	5	7	0
Medit	*Raphanus raphanistrum*	6	6	5	0	4	5
Species indifferent to association							
Cp	*Agropyron repens*	5	7	0	5	0	8
Eua	**Cirsium arvense**	10	8	0	0	0	7
Cosm	*Convolvulus arvensis*	5	7	6	4	7	0
Adv	**Erigeron canadensis**	8	7	0	4	0	7
Eua	*Lamium purpureum (Chenopodietea)*	2	7	0	5	7	0
Eua	*Medicago lupulina (Molinio-Arrhenatherea)*	2	7	5	4	8	0
Eua	*Melandrium album (Silene alba)*	2	8	5	4	0	7
Cosm	*Polygonum aviculare (Plantaginetalia)*	5	7	0	0	0	0
Cosm	*Stellaria media (Chenopodietea)*	5	6	0	4	7	8
Cosm	*Taraxacum officinale (Molinio-Arrhenatherea)*	2	7	0	5	0	7
	Σ		225	150	107	75	195
	Number of species		31	22	26	11	27
	$\bar{x}$		7.2	6.8	4.1	6.8	7.2

Table 7

Weed flora in row crops on drift-sand in the neighbourhood of Szeged (Kiskundorozsma, Jánosszállás, Sándorfalva, Szatymaz)
TIMÁR 1957
Association: Tribulo (terrestris)-Tragetum racemosi Soó et Timár

Life form	Flora element	Species	C	FO	T	HU	R	N
G	Cosm	*Equisetum ramosissimum*	I	8	7	4	7	4
Th	Eua	*Medicago lupulina*	I	7	5	4	8	0
Th	Pont-Med	**Tribulus terrestris ssp. orientalis**	III—V	9	9	3	8	4
Th	Medit	*Heliotropium europaeum*	I	8	8	4	8	6
Th	Eua	*Orobanche cumana*	0—III	—	—	—	—	—
H	Medit	*Diplotaxis tenuifolia*	I—II	8	7	3	0	4
Th	Eua	*Descurainia sophia*	I	8	6	4	0	6
Th	Cult	*Cucurbita pepo*	II—III	—	—	—	—	—
Th	Adv	*Erigeron canadensis*	I	7	0	4	0	6
Th	Cult	*Helianthus annuus*	III—V	—	—	—	—	—
H	Eua	*Chondrilla juncea*	I	8	7	3	8	0
Th	Cosm	**Portulaca oleracea**	IV—V	7	8	4	7	7
Th	Cosm	**Chenopodium album**	V	0	0	4	0	7
Th	Pont-Pann	*Corispermum nitidum*	I—II	9	9	2	7	4
Th	Eua	**Salsola kali** ssp. **ruthenica**	IV—V	9	7	0	7	8
Th	Cosm	*Amaranthus retroflexus*	II—III	9	9	4	0	9
Th	Adv	*Amaranthus chlorostachys*	I	9	9	5	7	9
Th	Adv	*Amaranthus albus*	II—III	9	9	3	0	7
Th	Cosm	*Polygonum aviculare*	I—III	7	0	0	0	0
Th	Cp	**Bilderdykia convolvulus**	III—V	7	8	0	6	6
Th	Cp	**Eragrostis pooides**	IV	8	7	3	0	4
G	Cosm	*Cynodon dactylon*	I—III	8	7	3	7	6
Th	Cosm	**Tragus racemosus**	III—IV	9	9	2	7	4
Th	Cosm	**Digitaria sanguinalis**	V	7	7	3	5	4
Th	Eua	*Setaria viridis*	III—V	7	6	4	0	7
Th	Adv	*Zea mays*	III—V	—	—	—	—	—
		Σ		169	147	66	99	112
		Number of species		21	19	19	14	19
		$\bar{x}$		8.0	7.7	3.5	7.1	5.9

C = constancy:
(percentage of surveyed areas on which the species occurs)

V = 81—100% constant species
IV = 61— 80% subconstant species
III = 41— 60%
II = 21— 40%
I = 1— 20%

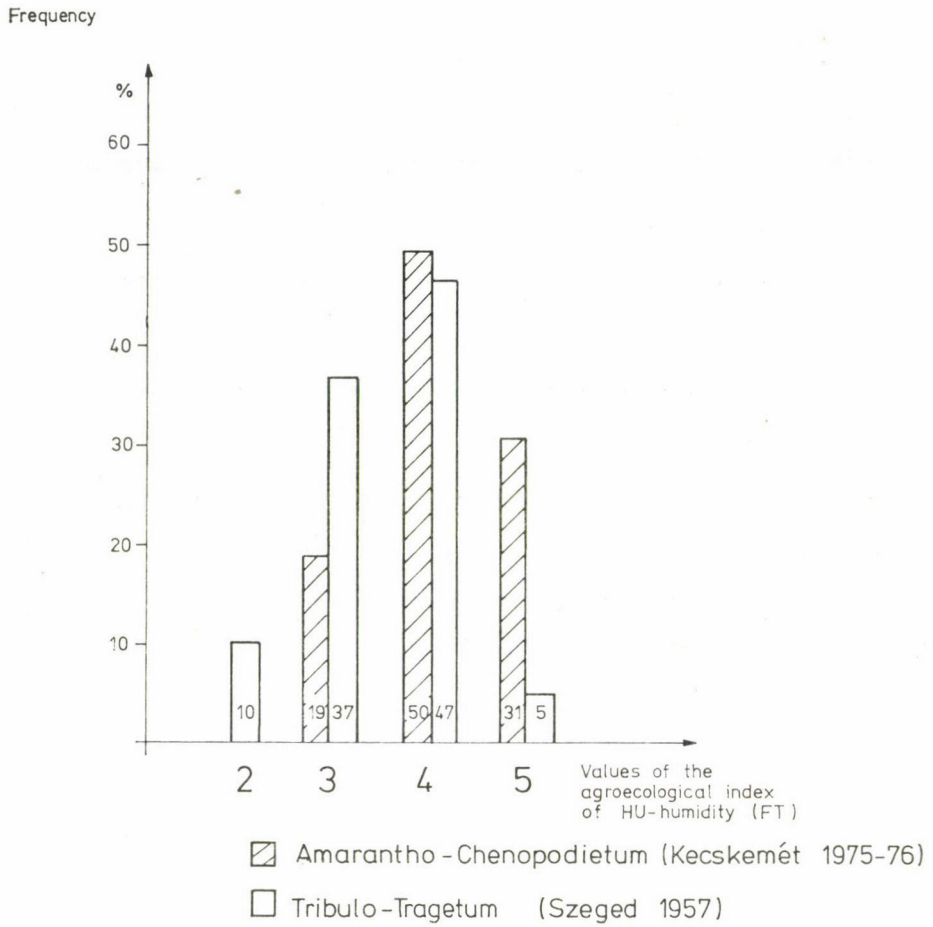

Fig. 4. Percentage frequency distribution of humidity (agroecological) indices for species of *Amarantho-Chenopodietum albi* (Soó) Kecskemét: Kisfái) and *Tribulo-Tragetum* Soó et Timár (Szeged) associations

On the basis of our surveying data differences between years and between surveying dates were compared for each species of the individual families (Table 5). The values characteristic of the families can be calculated by totalling the mean coverage data of the species. The highest mean coverage value was attained by the family *Portulacaceae*, followed by four families of national importance: *Compositae*, *Amaranthaceae*, *Chenopodiaceae* and *Gramineae*. (*Portulaca*, a species requiring higher temperatures and preferring light soils is always likely to be highly dominant in the Danube—Tisza mid-region.)

Some families, such as *Cannabiaceae*, *Malvaceae*, *Fabaceae*, *Solanaceae*, *Papaveraceae* and *Primulaceae*, occurred only in the first year, and even then only to a negligible extent. The family *Convolvulaceae*, on the other hand, only formed part of the weed flora from the second year of cultivation onwards.

The families *Cruciferae*, *Compositae* and *Gramineae* were associated with most species on the area.

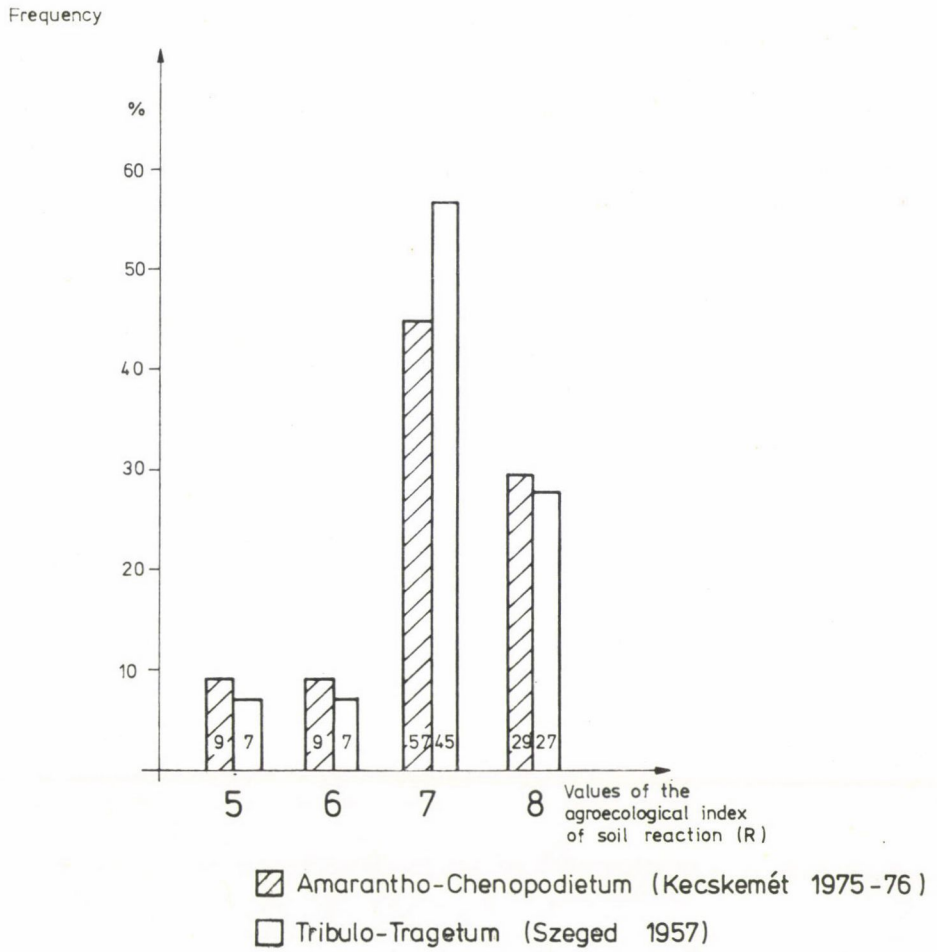

Fig. 5. Percentage frequency distribution of soil reaction (agroecological) indices for species of *Amarantho-Chenopodietum albi* Soó (Kecskemét: Kisfái) and *Tribulo-Tragetum* Soó et Timár (Szeged) associations

The agroecological indices of the species. In site investigations the weed species, like all other plants, can be regarded as bioindicators, since the present ecological grouping of the plant species (life form, flora element, aspect, stratum, etc.) and the establishment of their coenosystematic places and values serve to give a more exact analysis of the interaction between plant stands and environment.

In order to acquire a more exact knowledge of the site requirements within a shorter time, and to achieve a better utilization of plant indication data the species were placed in ecological groups. Earlier the demands for temperature (T), water (W) and soil reactions (R) were chiefly studied, so the values of the ecological factors determined for a species or association were called "TWR-values"; recently the *W* has been replaced by *F* in German literature.

Although the ecological systemization of plant species has been dealt with by a number of Hungarian authors it has not been used in practice in research on weed plants.

The agroecological indices of the weed species in a common amaranth — white goosefoot (fat-hen) association surveyed in the pear plantations were compared with surveying data obtained from the driftsand soils of south-eastern Hungary (Figs 3, 4, 5). Both the totalled data of the associations and the diagrams show quite clearly the differences between the site conditions of the two associations, though they are close to one another both geographically and as regards the abiotic factors. Nevertheless, several common features were also noted.

The weed species of the associations analysed were most responsive to light (FO), followed by water (HU). Approximately half the species were indifferent to the pH of the soil (R). According to the totalled data of *Amarantho-Chenopodietum albi* most of the weeds occurring in the association are heliophytes (FO = 7.2); they mostly grow in full light, though they tolerate the shade. The light demand of *Tribulo-Tragetum* is almost one unit higher (FO = 8.04). The temperature requirements of weeds in the pear orchards (though most of them demand heat) are lower (T = 6.8) than that of *Tribulo-Tragetum* (T = 7.7). On average the latter shows a sub-Mediterranean character.

As for water demand, the species of *Amarantho-Chenopodietum albi* represent a transition between drought-resistant plants and those with a medium water demand (HU = 4.1), while the association *Tribulo-Tragetum* indicates dry soils (HU = 3.5). The responses of the two associations to the pH and lime content of the soil are nearly identical. The soil of the *Amarantho-Chenopodietum albi* association, on the other hand, is indicated by the species to be richer in nitrogen (N = 7.2).

Finally, with reference to the frequency of weeds it should be noted that the number of species which occurs permanently in the young pear orchards is only 5 (15.6%): *Chenopodium album*, *Cirsium arvense*, *Cardaria draba*, *Digitaria sanguinalis* and *Bilderdykia convolvulus* (each of them belongs to a different family !). Another 5 species (15.6%), namely, *Amaranthus retroflexus*, *Descurainia sophia*, *Lamium amplexicaule*, *Portulaca oleracea* and *Erigeron canadensis*, which are only absent from the area in one or two cases, are also worth mentioning. It was noteworthy that *Ambrosia elatior*, which is a frequent weed elsewhere, occurred here only once.

*

Prepared at the Department of Botany of the University of Horticulture, Budapest.

A. Terpó, E. Dibuz

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BIOPOLYMER-METAL COMPLEX SYSTEMS. IX. PHYSICAL AND CHEMICAL PROPERTIES OF HIGH PURITY HUMIC ACIDS OBTAINED FROM HUNGARIAN BROWN COALS

Different humic substances (peat, lignite, nitrated brown coal, etc.) and products obtained therefrom are widely used today for the substitution of humus in soil improvement. Experience has shown that humic substances can be applied in agriculture not only as organic nutrients but also as stimulants for plant growth (LINEHAN 1976, VAUGHAN—MCDONALD 1976).

In the application of humic substances for soil improvement and plant production, however, considerable differences can be observed in the efficiency of the raw materials, which may be due to the different chemical and physical properties of humic substances and their main components: humic acids.

The present paper may be considered as a serial part of our previous extensive research into peat humic acids (LAKATOS *et al.* 1977a, b, c, MÁDY *et al.* 1979, MEISEL *et al.* 1979, SIPOS *et al.* 1978, VINKLER *et al.* 1976).

1. Gravimetric determination of the humic acid content. Gravimetric determination of the humic acid content of coals was carried out by classical methods (SCHNITZER—KAHN 1972).

2. Preliminary investigations of alkaline extraction. Low ash bitumen-free air-dry lignite II (30 g; crushed to min. 0.2 mm particles) was shaken with an aqueous solution of puriss. A. R. extractant (300 cm^3) for 1 week. After the addition of 100 cm^3 distilled water and centrifugation the mixture was filtered.

Humic acids were precipitated with approx. 30 cm^3 concentrated hydrochloric acid at pH = 0. The precipitate was allowed to stand for 2 days, and was then washed twice with distilled water (approx. 30 cm^3) and finally with hydrochloric acid (30 cm^3; 3%). The precipitate was dried at 110 °C.

Extraction was repeated twice with the insoluble residual coal by application of a 200 cm^3 extractant.

3. Preparation of raw lignite II humic acids. Low ash, bitumen-free lignite II (600 g) was suspended in 40 dm^3 water (pH = 3.5), then treated with an ultrasonic vibrator (ULTRON, 800 kW/sec.) for approx. 1 hour. The suspension was then adjusted with sodium hydroxide to pH = 10 and ultrasonic treatment was continued for approx. one more hour. By further addition of sodium hydroxide, the concentration of the solution was adjusted to 0.5 N (altogether 800 g puriss. A. R. NaOH). The suspension was mixed with the re-precipitated lignite II powder in a Biomix stirrer and the system was kept under constant stirring for two weeks. The mixture obtained was separated by centrifugation and the supernatant was filtered through a folding filter to remove possible coarse particles from the suspension. The pure alkaline solution was adjusted to pH = 1 with approx. 2 dm^3 concentrated hydrochloric acid and after sedimentation the brown humic acid precipitate was allowed to stand for 24 hours. The yellow solution (containing a fairly small amount of fulvic acid) above the precipitate was then removed by suction.

The residue (approx. 20 dm^3) was flushed (pH = 2) and the mixture was adjusted to pH = 0 with 0.5 dm^3 concentrated hydrochloric acid to prevent partial peptization of the

precipitate. The acidic system was allowed to stand for four days for further purification The supernatant was sucked off and the precipitate was separated by centrifugation. Washing was repeated twice and the suspension was kept under constant stirring for four days. The acidic solution was sucked off and after centrifugation the precipitate was washed until the onset of peptization, then dried at 110 °C to yield 60 g (10%) LH7.

4. Removal of grey humic acids from lignite II brown humic acids. A saturated sodium chloride solution (750 cm^3) was added to an aqueous solution (1%) of the LH7 sample, adjusted to pH = 11. The mixture was allowed to stand for 4 days and the precipitate (approx. 1%) was dried at 130 °C (LH7B).

5. Preparation of metal-free lignite II humic acids.

a) *Complexing by EDTA and purification on anion and cation exchange resins.* The disodium salt of ethylene diamine tetra-acetic acid [15 g Selecton B_2 (Komplexon III) mol. wt.: 372.24] was added to an aqueous solution (1%, 50 g)] of LH7B adjusted to pH-6—7. After stirring (for 5 days) and centrifugation of the solution, a slight amount of solid residue (0.1%) was obtained. Some of the residue was dissolved in distilled water and after filtration, the filtrates were combined with the supernatant.

The solution obtained was allowed to pass through a strong basic anion-exchange resin (Amberlite IRA-400 with hydroxyl cycle, capacity: 1.2 mequ./cm^3; column diameter: 80 mm, height: 700 mm) at a flow rate of about 500 cm^3/hr. Then the solution was passed through a strong acidic cation-exchange resin (Amberlite IR-120) with a direct connection to the Amberlite IRA-400, in hydrogen cycle (column diameter: 80 mm, height: 700 mm) at the flow rate given above. Pure lignite humic acid (LH7B1) was obtained from the column at pH = 2.6 in homogeneous colloidal solution, which was then evaporated and dried at 130 °C. Yield: 36 g, i.e. 72% LH7B1.

b) *Repurification by EDTA.* The aqueous solution (0.35%, 250 cm^3) of a LH7B1 sample was adjusted to pH-6—7. After the addition of Selecton B_2 (3 g), the solution was boiled for 15 min. and allowed to stand overnight. Purification was carried out on anion and cation exchange resins by the method described in point *a)* (LH7B2).

c) *Repurification by citric acid.* An aqueous solution (0.35%, 250 cm^3) of an LH7B1 sample was adjusted to pH = 12. After the addition of 3 g citric acid the solution was boiled for 15 min. and allowed to stand overnight. Purification was carried out on anion and cation exchange resins by the method described in point *a)* (LH7BC).

d) *Repurification by sodium fluoride.* The aqueous solution (0.35%, 250 cm^3) of LH7B1 was adjusted to pH = 12. After the addition of 2 g sodium fluoride, the solution was boiled for 15 min, and allowed to stand overnight. Purification was carried out on anion and cation exchange resins by the method described in point *a)* (LH7BF).

All the brown humic acid samples were prepared by this method.

6. Preparation of copper humate from purified lignite II humic acid. A few drops of copper sulphate solution were inoculated into the humic acid sample to form germs. Then an aqueous solution of 1 M copper sulphate (200 cm^3; pH = 4.5) was added to an aqueous solution of the humic acid sample (0.64%; 3500 cm^3) adjusted to pH = 5.5 with sodium hydroxide. The suspension obtained had pH = 4.0. By the addition of 0.1 N sodium hydroxide (1 dm^3) the suspension was adjusted to pH = 5. The system was allowed to stand for 1 week with daily stirring at pH = 5. The precipitate obtained was centrifuged and washed until no copper(II)-ion reaction could be detected. The sample was dried at 110 °C to yield 28 g CuLH7B1.

7. Derivatographic investigations. Investigations were carried out in an air stream with a MOM-type derivatograph. As the inert material, heated Al_2O_3 was used.

8. Molecular weight determination by analytical ultracentrifugation. The experiments were carried out as described by Sipos *et al.* (1978).

9. Molecular weight determination by gel filtration. Fractionation was carried out by gel

filtration on a Sephadex dextrane polymer (G-50 Fine, 20—80 micron; column diameter: 2 cm) by means of automatic fractionation (Czechoslovak model SF 62). The gel was prepared by allowing 10 g of the polymer to swell in 100 cm^3 distilled water for 24 hours with constant stirring. The gel was then poured into the column and simultaneously, distilled water was passed through the system at a flow rate of 30 cm^3/hr.

As the volume of gel showed no further change, the column was flushed with 0.2 N sodium hydroxide at the above flow rate at a given pH value. Then a given amount (a few mg) of the humic acid sample was dissolved in 2 cm^3 0.2 N sodium hydroxide and poured on the column. By automatic fractionation 5 cm^3 fractions were obtained at a rate of 30 cm^3/hr in the course of 8 hours.

Each fraction was examined using a Zeiss Spekol spectrophotometer to determine the amount of absorbance. Then, on the basis of previously determined calibration curves, the corresponding concentration values could be calculated. With a knowledge of these values and the exact amount of initial humic acids, the % amount of each humic acid sample in 5 cm^3 fractions could be calculated.

Molecular weight values of the individual fractions were determined by the following molecular weight standards:

8 500 D trypsin inhibitor I
10 000 D dextrane D10
12 500 D cytochrome C
17 500 D myoglobin (10)
21 100 D trypsin inhibitor II
25 000 D chymotrypsin inhibitor

and finally, a homogeneous pure peat humic acid fraction was determined by ultracentrifugation (SIPOS *et al.* 1978).

With a knowledge of the % amount and the molecular weights of the individual fractions, the differential molecular weight distribution curves were plotted.

The aggregation effect of copper(II) ions has also been studied. For this purpose, dilute 0.125% humic acid solutions were mixed with (0.01 mole) copper(II) chloride solutions of equivalent volume. Molecular weight determinations were carried out at copper(II) ion concentrations immediately preceding the value of coagulation where the copper(II) humate formed was still in solution. The pH values of the copper(II) humate were adjusted to the original pH values of the humic acid samples. Gel filtration examinations were carried out at pH = 7.5.

10. IR absorption spectra. The samples were dried at 130 °C, mixed with potassium bromide, stirred in a vibrator for 3 minutes and compressed into discs. IR absorption spectra were recorded on a UR-10 spectrophotometer.

11. Acid-alkalimetric titration. The total acid number of the humic acid samples was determined by slow 0.1 N NaOH titration, in the course of which, changes in the pH values were continuously recorded by a glass electrode (Radelkisz OP-205 type pH-meter).

12. Determination of the metal content of samples. The metal content and metal-ion bonding capacity of the samples were studied by complexometry (MEISEL *et al.* 1979) or on a Varian Techtron AA5 type atomic absorption spectrophotometer.

Characterization of raw and purified coals with reduced bitumen and ash content

The main aspects in selecting the raw materials as humic acid sources were to find coals with potentially rich resources from different geological ages. For this purpose the following coal types were selected: breeze lignite I (pliocene; Gyöngyösvisonta), coarse lignite II (miocene; Cser-shaft, Várpalota) and screened brown coal (eocene: Dudar).

Our investigations have been extended to the study of synthetic compost prepared from lignite II in the presence of sulphuric and phosphoric acid by means of nitric acid oxidation and nitration (hereafter: nitrated lignite II), in order to compare natural and regenerated humic acids.

Characteristic analytical data of the different types of raw coals are collected in Table 1 and ash composition data are shown in Table 2.

Most inorganic contaminations were found in lignite I, which contained 36.2% ash with a considerable amount of SiO_2 (51.8%), Fe_2O_3 (12.8%) and Al_2O_3 (16.0%). The SiO_2 and Fe_2O_3 content of the ash of brown coal (Dudar) was found to be much lower, showing rather limy properties and some Al_2O_3 content. The lowest ash (14.3%) was detected in lignite II

Table 1

Analytical date of raw coal samples

Coal types	Lignite I (Gyöngyös-visonta)	Lignite II (Cser-shaft, Várpalota)	Brown coal (Dudar)	Nitrated lignite II (Cser-shaft)
Geological age	pliocene	mid-miocene	lower eocene	—
Data calculated for moisture-free sample:				
Ash, %	36.2	14.3	19.5	33.3
S (total), %	3.0	2.5	5.1	6.3
N, %	0.6	0.6	0.7	4.8
P_2O_5, %	—	0.29	0.53	4.28
Data calculated for moisture- and ash-free sample:				
Volatile, %	59.2	53.4	56.2	—
C, %	62.2	65.7	67.5	47.2
H, %	5.6	4.9	5.5	4.5
S (organ), %	2.2	2.4	5.8	2.6
N, %	0.9	0.7	0.9	3.4*
O (calculated), %	29.1	26.3	20.3	42.3
O + 1/2 S, %	30.2	27.5	23.2	43.6
H/C atom	1.08	0.90	0.98	1.14
O/C atom	0.36	0.31	0.26	0.69
Humic acid, %	38.8	26.4	87.7	69.6
Total OH, mequ./g	3.72	4.78	3.70	7.79
Carboxyl OH, mequ./g	2.17	1.06	0.96	2.64
Phenolic OH, mequ./g	1.55	3.72	2.74	5.15
Methoxyl OCH_3, mequ./g	1.31	0.63	0.07	0.39

* N bound to carbon.

Table 2

Ash composition of raw coal types

Coal type	Lignite I (Gyöngyös-visonta)	Lignite II (Cser-shaft, Várpalota)	Brown coal (Dudar)	Nitrated lignite II (Cser-shaft)
SiO_2, %	51.8	12.70	27.1	11.5
Fe_2O_3, %	12.8	9.30	5.4	3.2
Al_2O_3, %	16.0	7.40	15.4	2.5
CaO, %	7.6	30.5	20.5	25.9
MgO, %	2.5	8.0	4.5	1.8
K_2O, %	1.8	0.68	1.0	11.5
Na_2O, %	0.3	0.37	0.4	0.6
TiO_2, %	0.6	0.9	0.8	0.2
SO_3, %	6.3	30.8	20.5	18.3

with relatively low Al_2O_3 content but a considerable amount of CaO. The great amount of ash, which was composed mainly of gypsum, phosphates, nitrates and potassium chloride and derived from nitrated lignite II, was formed in the course of synthetic compost production.

Volatile values calculated for pure carbon unanimously showed the lowest degree of coalification in lignite I, derived from the pliocene period. The volatile value for brown coal (eocene, Dudar), however, appeared to be higher than expected on the basis of coalification, which may be assigned to the considerable amount of bitumen in the coal.

The humic acid content of brown coal (eocene, Dudar) determined by extraction with aqueous sodium hydroxide was very high, approx. 88% calculated for moisture-free and ash-free raw materials. The amount of humic acids extracted from lignites was, however, much lower (approx. 30%) although it increased in nitrated lignite II to about 70%, probably due to the digestion of insoluble humic substances in lignite II.

The amount of carboxylic and phenolic hydroxyl groups determined by $Ba(OH)_2$ or $Ba(CH_3COO)_2$ and the methoxyl content measured by the Zeisel method showed values as expected. As methoxyl groups were the first to split off in the course of coalification, the amount of these groups could be determined as follows: 1.31 mequ./g for lignite I, compared to 0.63 mequ./g found in lignite II and 0.07 mequ./g detected in brown coal (Dudar). Carboxyl groups were found to split off more slowly and phenolic hydroxyl groups seemed to be the most stable. In accordance with this, more carboxyl than phenolic-hydroxyl groups could be detected in lignite I while the opposite could be observed for lignite II and brown coal, where carboxyl groups were only 1/3 of the amount of phenolic-hydroxyl groups.

The data of functional groups of nitrated lignite II do not fit in the order of coalification. As a result of nitric acid treatment some of the methoxyl groups split off, involving an increase in the number of acidic functional groups and metal-bonding capacity.

In order to obtain high purity humic acids, as the first step the ash content of raw coals was reduced by treatment with 10% HCl for 24 hours. The partly hydrolysed coal samples were then freed from bitumen by extraction with a 3 : 1 benzene-alcohol solvent mixture in a Soxhlet apparatus for 48 hours.

Analytical data and ash composition of the raw materials with reduced bitumen and ash content are given in Tables 3 and 4.

Table 3

Analytical data of coal samples

Coal types		Ash	S	N	Volatile	C	H	S_x
		%						
		Calculated for moisture-free sample			Calculated for moisture-			
Lignite I (Gyöngyösvisonta)	1	30.0	3.2	0.6	55.6	61.1	5.5	2.2
	2	30.3	3.6	0.6	55.3	61.7	5.5	2.2
Lignite II (Cser-shaft)	1	3.9	2.6	1.1	49.2	63.0	4.4	2.4
	2	4.2	2.6	0.8	49.5	65.2	4.4	2.4
Brown coal (Dudar)	1	10.4	6.2	0.8	51.7	67.2	5.4	5.8
	2	11.2	5.8	0.8	50.3	67.0	5.2	5.8
Nitrated lignite II (Cser-shaft)	1	30.1	6.3	2.1	—	51.7	3.9	2.6
	2	30.3	6.7	2.1	—	54.2	4.3	2.6

Table 4

Ash composition of coals with reduced bitumen and ash content

Coal types		SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO	K_2O	Na_2O	TiO_2	SO_3
		%								
Lignite I (Gyöngyösvisonta)	1	60.6	13.8	17.7	2.3	1.4	1.4	0.3	0.7	0.5
	2	60.6	14.3	17.8	2.4	1.3	1.5	0.3	0.7	0.4
Lignite II (Cser-shaft)	1	—	—	—	—	—	—	—	—	—
	2	—	18.8	12.3	13.3	3.2	—	—	—	—
Brown coal (Dudar)	1	54.1	9.3	25.9	2.5	1.0	1.8	0.2	1.2	0.8
	2	53.5	9.4	26.6	2.6	1.0	1.6	0.3	1.2	1.1
Nitrated lignite II (Cser-shaft)	1	15.7	1.4	2.0	31.4	0.3	0.9	0.4	0.1	39.0
	2	18.1	1.6	2.1	31.2	0.3	1.0	0.3	0.2	38.0

1 = product of treatment with 10% hydrochloric acid
2 = product obtained by extraction with benzene-alcohol (3 : 1)

A considerable amount of ash could be removed in the case of limy samples (lignite II, brown coal). The substantial weight loss observed for nitrated lignite II (synthetic compost) points to the solution of potassium chloride and ammonium salts as well as water-soluble phosphates and fulvic acids, the latter being formed as the by-product of nitration.

In the course of benzene-alcohol extraction, the weight loss was found to be the highest for brown coal, which may be due to its high bitumen content. It should be noted, however, that with benzene-alcohol extraction the removal of bitumen may also involve the extraction

with reduced bitumen and ash content

N	O	O + 1/2 S	H/C atom	O/C atom	Humic acid, %	Weight loss by treatment with	
%						hydrochloric acid	benzene-alcohol
1 d ash-free sample						%	
0.9	30.3	31.4	1.08	0.38	33.5	6.7	—
0.9	29.7	30.8	1.07	0.37	25.2	—	5.0
0.8	29.4	30.6	0.84	0.36	28.7	8.4	—
1.1	26.9	29.1	0.81	0.33	27.8	—	8.7
0.9	20.7	27.9	0.96	0.31	85.2	12.6	—
0.9	21.1	24.0	0.93	0.27	66.2	—	12.5
3.2	38.6	39.9	0.93	0.58	75.7	32.8	—
3.0	35.9	37.2	0.95	0.51	75.3	—	11.0

1 = product of 10% hydrochloric acid treatment
2 = product after extraction with benzene-alcohol (3 : 1)

of small mol. weight fulvic acids, which generally leads to a decrease in the humic acid content of the extracted samples.

These effects can be detected only to a small degree in the analytical data of pretreated raw materials (Table 3); the decrease in the H/C ratio as related to the starting materials in the case of lignite II and brown coal, however, unanimously points to the removal of bitumen. The decrease of the O/C ratio in the case of nitrated lignite II, on the other hand, shows that fulvic acids containing several oxygen functional groups have been washed out of the mixture.

Ash composition data (Table 4) do not only reflect the characteristics of ash, which belongs primarily to the organic carbon substances, but also the amount of secondary (inorganic) incorporations and tertiary inorganic components (rocks involved in the mining process). Ca^{2+}-ions can be most readily removed from samples with lower ash content and less inorganic contaminations by acid, while silicon, iron and aluminium are bound to organic substances and are, thus, present in the ash in a higher percentage.

After hydrochloric acid treatment and benzene-alcohol extraction of the raw coals, structural changes were followed by infra-red absorption spectroscopy for lignite II, nitrated lignite II and brown coal (Fig. 1, 2, 3).

In the spectrum of untreated lignite II (Fig. 1) the ν (CH_3) and ν (CH_2) bands of paraffin hydrocarbon at 2926 and 2853 cm^{-1} point to the presence of bitumen, wax and resins. No bands of amide I and II ranging from 1660 cm^{-1} to 1540 cm^{-1} characteristic of peats can be observed, however, which is in agreement with the analytically determined nitrogen values (peat N: 3—4%, lignite N: 1%) (LAKATOS *et al.* 1977b, VINKLER *et al.* 1976).

After treatment with hydrochloric acid ν(C=O) stretching vibration bands of the carboxylic group could be recorded between 1710—1730 cm^{-1}. In the IR spectra of the raw materials, the band between 1590—1600 cm^{-1} is slightly shifted into the region between 1610—1630 cm^{-1} due to the antisymmetric $\nu_{as}(COO^-)$ stretching vibrations of the metal-carboxylate group (calcium-iron-aluminium humate). This band may be assigned to the semiquinone-type CO...H group bound by a hydrogen bridge and to aromatic stretching of the C=C bond, as

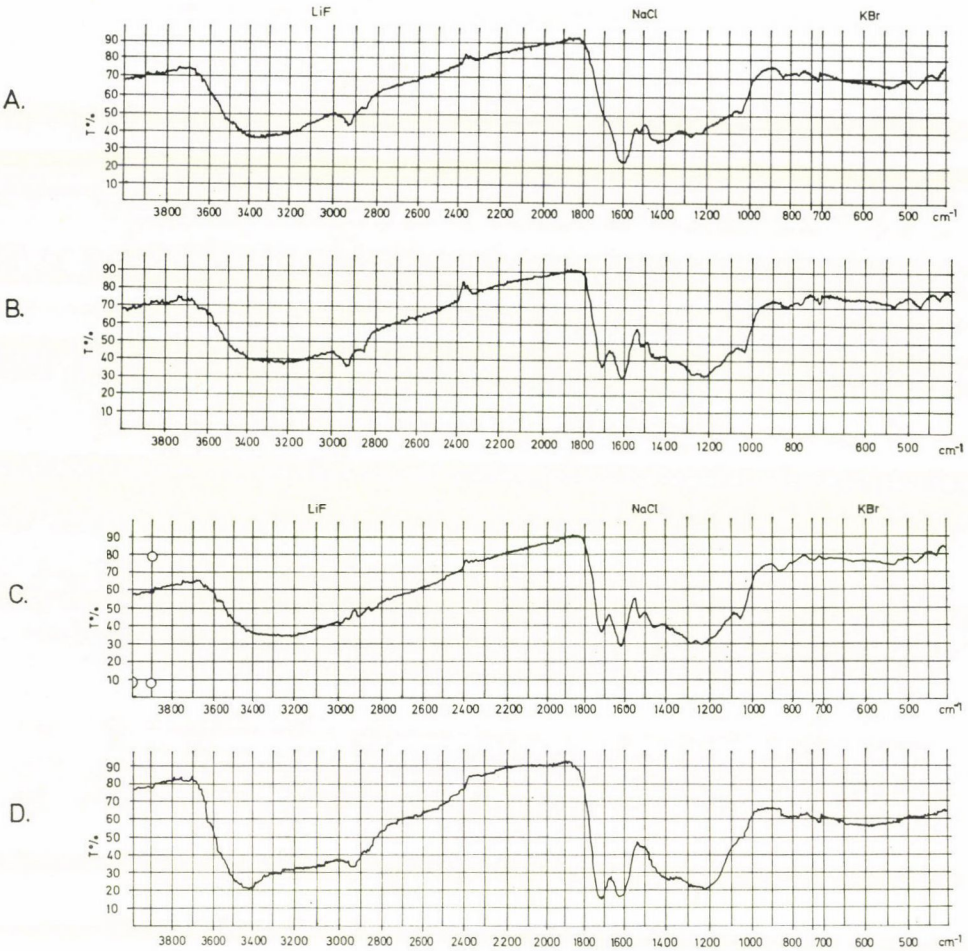

Fig. 1. IR spectra of lignite II derivatives (Várpalota, Cser-shaft). *A)* Raw lignite II; *B)* treated with HCl; *C)* treated with benzene-alcohol mixture; *D)* humic acid, purified with Selecton B_1, anion and cation exchange resins: LH-7B1

well as to the decrease in the amount of calcium and the increase in aluminium(III) and iron(III).

Benzene-alcohol treatment removes most of the extractable bitumen (the intensity of bands between 2926 and 2853 cm^{-1} decreases), the band corresponding to aromatic skeletal vibrations at about 1510 cm^{-1}, however, points to the presence of residual non-extractable bitumen substances and coal matrix in lignite II.

In the IR spectra of synthetic compost samples containing nitrated lignite II (Fig. 2), the carboxylate band is shifted from 1590 cm^{-1} to 1620 cm^{-1}, which may be assigned to the fact that during the technological process (nitric acid oxidation and nitration in the presence of sulphuric and phosphoric acid), the Ca-humate of lignite II was converted into calcium salts (sulphate, nitrate and phosphate) and aluminium and iron humate residues could be detected in the sample.

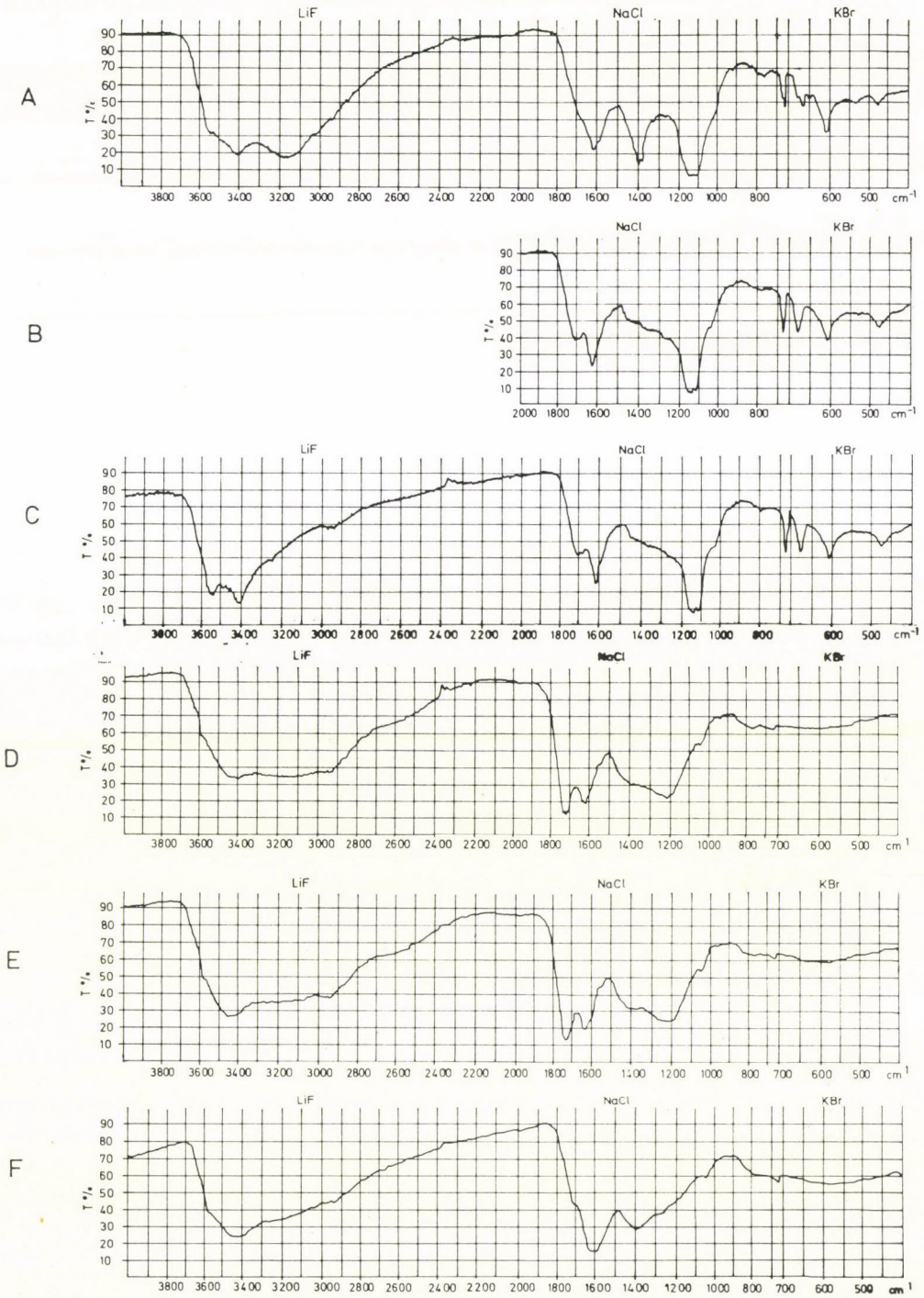

Fig. 2. IR spectra of nitrated lignite II derivatives. *A)* Raw nitrated lignite II (synthetic compost): LHN-1; *B)* LHN-1 treacted with HCl: LHN-2; *C)* LHN-2 extracted with benzene-alcohol mixture: LHN-3; *D)* raw nitrohumic acid: LHN-4; *E)* nitrohumic acid purified with NaF, anion-cation exchange resins: LHN-4F; *F)* Cu-humate: Cu-LHN-4

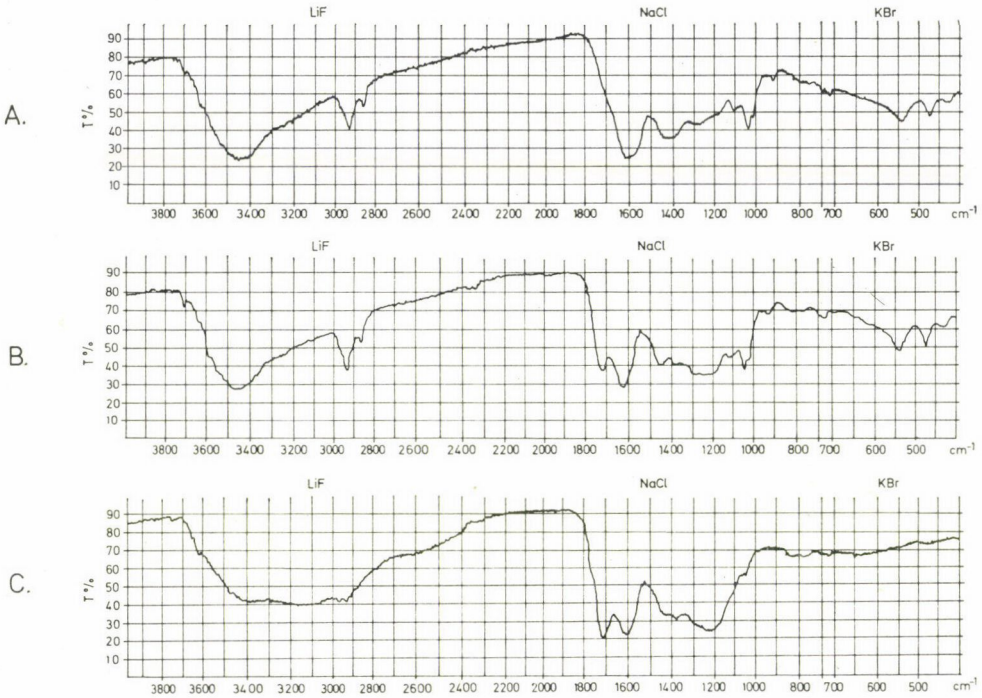

Fig. 3. IR spectra of brown coal derivatives (Dudar). *A)* Raw brown coal: SZHD-1; *B)* SZHD-1 treated with HCl: SZHD-2; *C)* humic acid purified with NaF, anion and cation exchange resins: SZHD4-F

The bands assigned to inorganic salts can be discerned in the spectrum as follows: ν_3 and ν_4 vibrations of sulphate can be found in the region between 1100—1160 cm^{-1} and at 620 cm^{-1}, respectively; the ν_3 vibration of nitrate at about 1400 cm^{-1} is split into two bands, with the narrower band pointing to the presence of ammonium salt (ammonia was used in the preparation of the synthetic compost for neutralization of the product). The ν_3 vibration characteristic of phosphate can be observed at 1080 cm^{-1} while the νOH vibration of phosphoric acid is recorded at approx. 3200 cm^{-1}.

After hydrochloric acid and benzene-alcohol treatment of nitrated lignite II, the intensity of the carboxylic ν(C=O) band at 1730 cm^{-1} increased, whereas the band intensity at 2926 cm^{-1} and 2853 cm^{-1} characteristic of extracted bitumen was found to decrease. The band at 1510 cm^{-1} corresponding to aromatic frequency vibrations was, however, less intensive relative to that of raw lignite II which points to partial decomposition of the non-extractable bitumen (coal matrix) due to nitric acid treatment.

In the case of brown coal (Fig. 3) the spectra of both untreated samples and those treated with hydrochloric acid, $\nu(CH_3)$ and $\nu(CH_2)$ (stretching vibrations) of the paraffin hydrocarbon at 2926 cm^{-1} and 2853 cm^{-1} show much higher band intensity, which points to a higher amount of bitumen in the coal.

The intensity ratios of the bands at 1720 and 1620 cm^{-1} show a trend similar to the above after treatment with hydrochloric acid. The band at 1050 cm^{-1} due to ν(C—O), ν(C—O—C) and ν(Si—O—H) vibrations, is most intensive in the case of brown coal. No decrease in intensity could be observed after treatment with hydrochloric acid.

Extraction and purification of humic acids

In order to obtain an extractant giving optimum yield, we carried out experiments with different alkaline solutions (LAKATOS *et al.* 1977b) for lignite II. The extractions were repeated three times and the weight of extracted humic acids as well as the ash content were determined. According to the data of Table 5, the sodium pyrophosphate solution, which was

Table 5

The effect of different extractants in the extraction of humic acid from lignite II (Cser-shaft)

Sample	1	2	3	4	5	6
Extractant	0.5 N NaOH	0.5 N $Na_4P_2O_7$	0.5 N NaOH + $Na_4P_2O_7$	0.5 N NaOH + 0.1 M EDTA (1 : 1)	0.5 N NaOH and vibr. treatment	0.5 N KOH + 0.1 M citric acid
Humic acid extracted	2.81 0.574 0.360	0.10 0.02 0.01	1.18 0.80 0.712	0.3 1.466 0.01	3.16 0.20 0.01	0.156 0.035 0.020
g	3.744	0.13	2.692	1.776	3.37	0.111
%	12.5	0.43	9.0	5.92	11.2	0.37
Ash, %	3.17	3.91	3.88	3.87	1.31	5.87

found to be most effective in the extraction of lowland peat (pH = 11), proved to be the poorest extractant besides the mixture of aqueous solutions of potassium hydroxide and citric acid. The most effective extractant appeared to be the aqueous solution of 0.5 N sodium hydroxide.

Experiment No. 5 was carried out by a different method: the sample (30 g) was first suspended in distilled water (2000 cm^3), then treated with an ultrasonic generator (ULTRON; 800 kW/sec.) for 15 minutes. After the addition of sodium hydroxide, the mixture was agitated overnight. In this experiment most of the humic acids were already dissolved after the first extraction and the above treatment gave the product with the lowest ash content (1.31%).

In order to obtain sufficiently high yields, large scale alkaline extractions were carried out, which required, in addition to ultrasonic treatment, intensive agitation (8 hr/day) for 2 weeks.

After centrifugation of the alkaline solution, humic substances were separated by hydrochloric acid treatment, then washed and flushed three times.

Grey humic acids were separated from raw brown humic acids in an alkaline medium (pH = 10.9) at the isoelectric point by the addition of sodium chloride in order to eliminate blocking of the resin in further ion-exchange purifications.

Raw brown humic acids [indicated in Table 6 as (HS)] still retain a considerable amount of ash (1.3—7.4%). Further purifications were required, therefore, to reduce the amount of complexing Al(III) and Fe(III) contaminations, which are most disturbing in structural investigations.

As is well known, ethylene-diamine-tetraacetate (EDTA) in aqueous solution forms a very stable complex with various metal ions. A treble amount of excess EDTA was cal-

Table 6

Reduction of amount of aluminium and iron in the purification of raw humic acid

Initial raw coal type		Lignite I (Gyöngyös-visonta)	Lignite II (Cser-shaft)	Brown coal (Dudar)	Nitrated lignite II (Cser-shaft)
Ash (dry), %					
SZ		36.2	14.3	19.5	33.3
HS		7.37	1.27	5.45	1.45
THS		2.68	0.66	1.54	0.88
Ash analysis:					
SZ	Al_2O_3, %	16.0	7.4	15.4	25.9
	Fe_2O_3, %	12.8	9.3	5.4	3.2
HS	Al_2O_3, %	25.2	25.3	8.0	20.0
	Fe_2O_3, %	5.1	9.3	1.2	4.3
THS	Al_2O_3, %	20.7	21.4	10.0	10.9
	Fe_2O_3, %	3.6	4.8	1.4	2.1
Al	10^{-2}, %				
	SZ	306.0	55.7	159.0	44.2
	HS	98.2	16.9	22.8	15.3
	THS	29.2	7.45	8.17	5.08
Fe	10^{-2}, %				
	SZ	325.0	93.5	74.0	75.0
	HS	26.3	4.83	4.46	4.37
	THS	6.78	2.21	1.47	1.29
Al	mequ./g				
	SZ	11.3	0.206	0.592	0.163
	HS	0.363	0.063	0.085	0.057
	THS	0.108	0.028	0.030	0.019
Fe	mequ./g				
	SZ	0.583	0.167	0.131	0.133
	HS	0.065	0.009	0.008	0.010
	THS	0.012	0.004	0.003	0.002
Al	atom				
Fe	SZ	19.4	1.2	4.4	1.2
	HS	5.6	7.0	10.5	5.7
	THS	9.1	7.0	10.0	9.6

SZ = raw coal, HS = raw humic acid, THS = purified humic acid.

culated and used relative to the total ash content, which was considered, for the sake of simplicity, to be pure aluminium oxide. EDTA and contaminating metal-EDTA anion complexes are bound on strong basic resins while the side-ions (in this case sodium ions) can be bound on strong acidic resins in hydrogen cycle. By this combined procedure (LAKATOS *et al.* 1977b) the 1.27% ash of raw humic acid extracted from raw lignite II could be reduced to approx. 0.9%.

In the IR spectrum of the purified sample LH7B1 (Fig. 1D) it may be observed that the intensity of the 1720 cm^{-1} band characteristic of carboxylic groups has considerably increased in relation to that of the carboxylate band at 1620 cm^{-1}. The shoulder at 1630 cm^{-1}, however, which is mainly due to antisymmetric vibrations of aluminium carboxylate, suggests further aluminium contaminations.

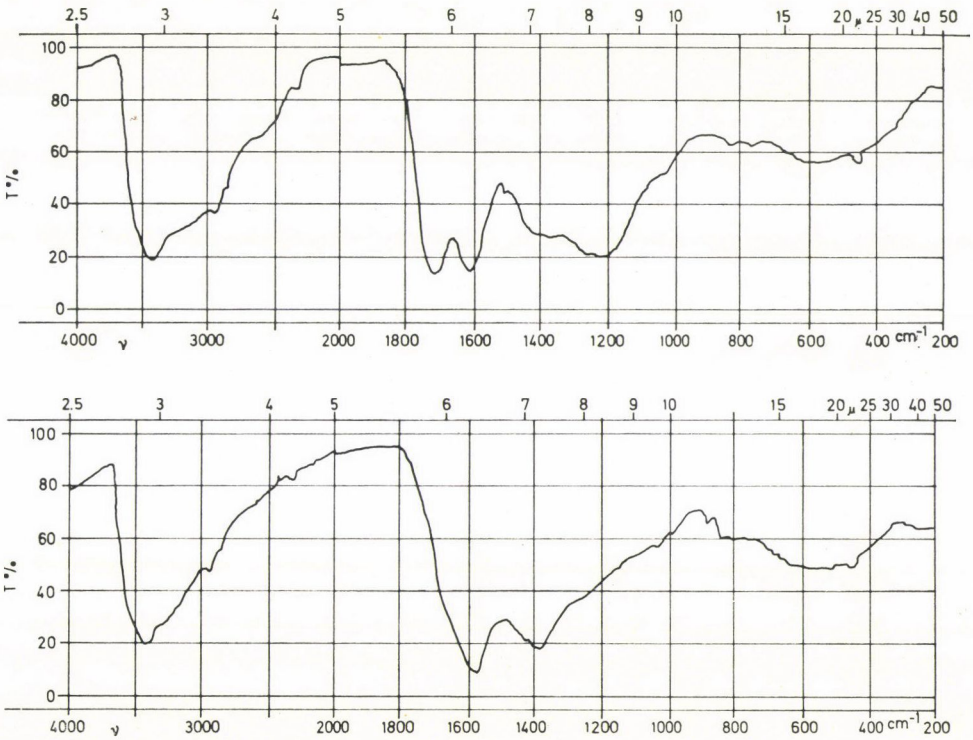

Fig. 4. IR spectra of lignite II derivatives (Cser-shaft). *A)* Humic acid sample repurified with Selecton B_1, anion and cation exchange resins: LH-7B2; *B)* Na-humate: NaLH-7B1

Further reduction of the metal ions (mainly aluminium contaminations) has been approached by three methods: boiling with aqueous solutions of

1. EDTA, or
2. citric acid, or
3. sodium fluoride,

followed by purification with anion and cation exchange resins as described above.

IR absorption spectra of the humic acid samples (LH7B2, LH7BC, LH7BF) showed on repeated purification a further reduction in the aluminium content (Fig. 4A and 5A, B). According to the analytical data, the 0.90% ash content of LH7B1 humic acid was reduced to 0.66% after treatment with an aqueous solution of sodium fluoride. This may be explained by the higher stability of aluminium fluoride complexes (log K = 19.24) compared to EDTA complexes (log K = 16.13).

For the elimination of metal traces in further samples purification was carried out by a combined method of complexing with an excess amount of sodium fluoride and treatment with anion and cation exchange resins.

Humic substances purified by the combined method (THS) contain only 0.66—2.6% ash, as can be seen in Table 6. It may be established from the trend of Al(III) and Fe(III) contaminations that approximately 2/3 of the above metal contaminations can be eliminated by complexing and treatment with ion exchange.

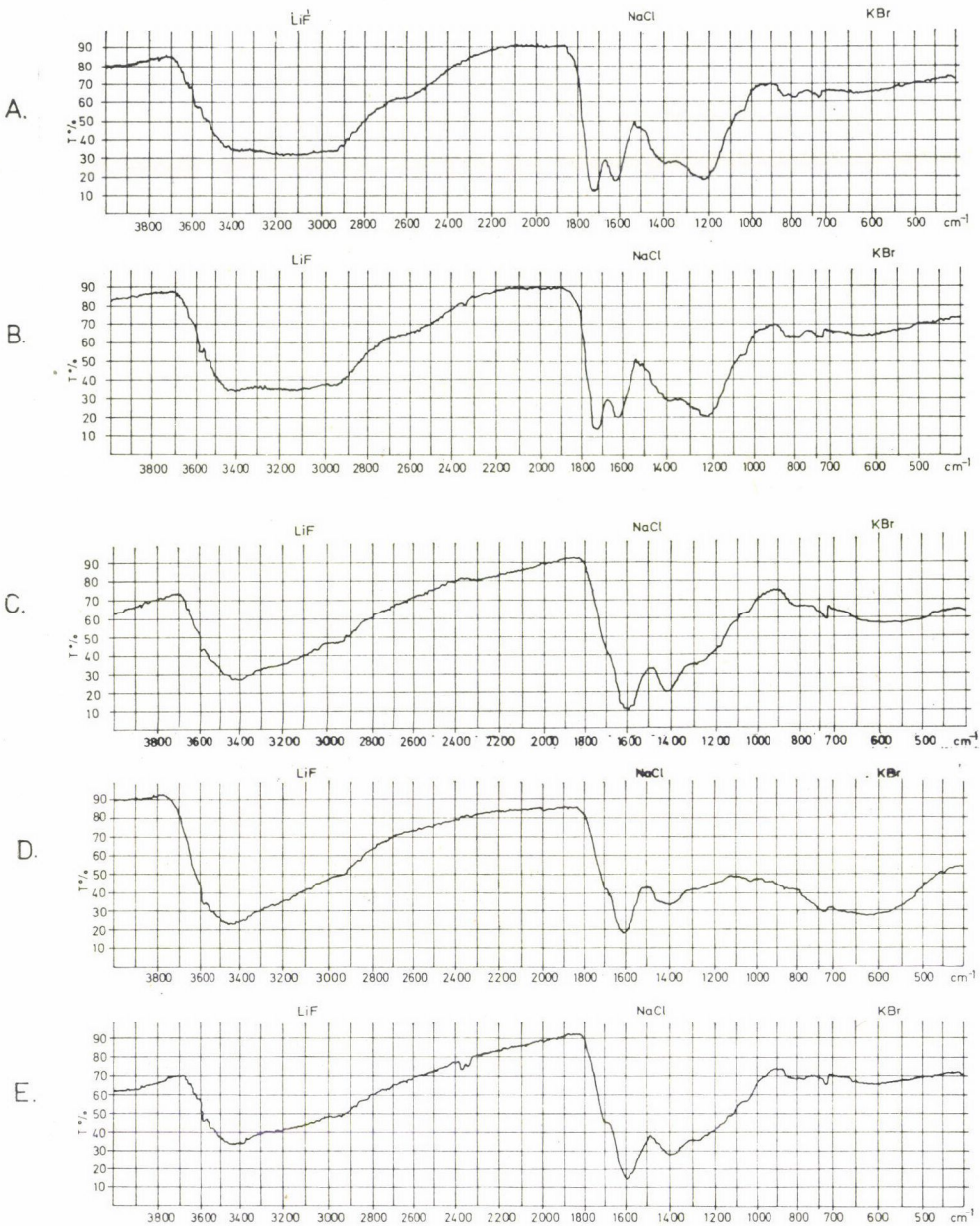

Fig. 5. IR spectra of lignite II derivatives (Cser-shaft). *A)* Humic acid purified by citric acid and anion and cation exchange resins: LH-7BC; *B)* Humic acid purified with NaF and anion-cation exchange resins: LH-7BF; *C)* Ca-humate: CaLH-7B2; *D)* Al-humate: AlLH-7B2; *E)* Cu-humate: CuLH-8B1

Finally, it may be stated that our purified humic acid samples (with the exception of lignite I with its originally high ash content) contain only 10^{-2} % aluminium and iron.

In high purity humic acids the amount of Al(III) atoms is approximately ten times as high as that of Fe(III) atoms (Table 6), which may be explained by the higher complexing affinity of the Al(III) ion.

Characterization of purified humic acids

Elementary composition

Humic substances are analysed mainly on the basis of elementary composition. As could be observed in Tables 1 and 7, the O/C ratios are higher in humic acids than in raw coals, which suggests a concentration of oxygen functional groups in humic acids. The only exception is nitrated lignite II, where the high ratio of O/C is due to the higher number of oxygen functional groups in the fulvic acids formed in the course of nitric acid treatment. After the extraction of humic acids, however, these fulvic acids are washed out as water soluble products.

The O/C ratios of humic acids are closer to the O/C values of raw coals, which points to substances with similar composition. In this case again, however, classification according to geological age and degree of coalification is unambiguous.

Table 7

Analytical data of purified humic acids

Initial raw coal type	Lignite I (Gyöngyös-visonta)	Lignite II (Cser-shaft)	Brown coal (Dudar)	Nitrated lignite II (Cser-shaft)
Ash (dry), %	2.68	0.66	1.54	0.88
Data calculated for moisture- and ash-free samples:				
C, %	61.8	62.6	63.6	59.0
H, %	4.3	3.6	3.6	3.5
S, %	2.2	2.4	5.8	2.6
N, %	1.5	1.6	1.1	3.4
O (calculated), %	30.2	29.8	25.9	31.5
O + 1/2 S, %	31.3	30.0	28.8	32.8
H/C atom	0.84	0.69	0.68	0.72
O/C atom	0.38	0.36	0.31	0.42
Total OH, mequ./g	7.10	8.24	6.34	8.87
Carboxyl OH, mequ./g	4.28	4.62	3.62	5.50
Phenol OH, mequ./g	2.82	3.62	2.72	3.37
Methoxyl OCH_3, mequ./g	0.51	0.22	0.00	0.21
Humic acid, pH, mequ./g	3.4	2.6	3.9	2.7
Acid number (Acidometric titration), mequ./g	5.50	5.70	5.0	6.75
Cu(II) bonding (complexometric), mequ./g	1.75	3.40	1.50	2.25
Al 10^{-2}, %	29.20	7.45	8.17	5.08
Fe 10^{-2}, %	6.78	2.21	1.47	1.29

The highest O/C value was found in the case of nitrated lignite II, which is partly due to oxidation with nitric acid and partly to the formation of organic nitro groups (see high N: 3.4%). The H/C ratios are lower for humic substances than for raw coals, which suggests that the bitumen extracts with high hydrogen content have been removed from the humic acid polymers in the course of benzene-alcohol extraction and saponification with sodium hydroxide.

In the case of bitumen-free humic acids, the order of coalification can be unanimously determined on the basis of H/C ratios.

Infrared spectra

The IR absorption spectra of purified humic acids show similar features, although closer investigation leads to the following results: (Fig. 2E LHN4F; Fig. 3C SZHD4F; Fig. 5B LH7BF; Fig. 6 LHGY4F).

The intensity of the $\nu(CH_3)$ and $\nu(CH_2)$ bands characteristic of paraffin hydrocarbons determined at about 2926 cm^{-1} and 2853 cm^{-1}, which suggests the presence of bitumen extract, decreased substantially as the result of treatment with NaOH (e.g. brown coal SZHD4F).

In the IR spectra of humic acids it may be well observed that the band of $\nu(C=O)$ frequency vibrations of the carboxylic group in the range between 1710 —1730 cm^{-1} is

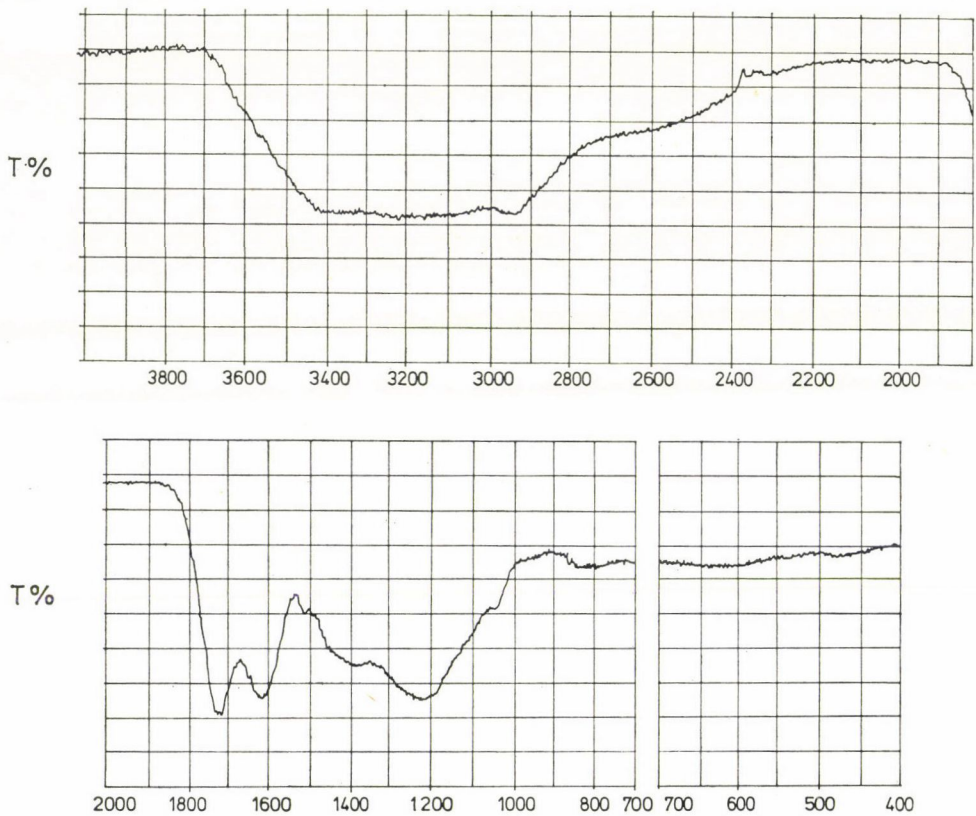

Fig. 6. IR spectra of humic acid extracted from lignite I and purified with NaF and anion-cation exchange resins

much more intensive than that of the antisymmetric $\nu_{as}(COO^-)$ frequency vibrations of the carboxylate group in the 1590—1600 cm^{-1} range. The latter is shifted, however, to the range between 1615—1630 cm^{-1} mainly due to the frequency vibrations of the keto groups in the hydrogen bridge bond.

The IR spectrum of nitrated lignite II (LHN4F) reveals an exceptionally high number of carboxyl groups due to nitric acid treatment.

The 1510 cm^{-1} band may be assigned to the skeletal C=C frequency vibrations of the aromatic ring, which suggests, among others things, non-extractable bitumens. After extraction with a mixture of benzene-alcohol the absorption observed at this band could still be detected, e.g. in the case of lignite II (Fig. 1C). Humic acid extraction by sodium hydroxide, however, removes even these bitumens, as is evident from the intensity decrease at 1510 cm^{-1} in the spectrum of lignite II LH7B1 humic acid (Fig. 1D). It is noteworthy that in the IR spectrum of the humic acids (SZHD4F) of brown coal, which shows a higher degree of coalification, no such absorption band can be observed.

In the IR spectra of nitrated lignite II (Fig. 2E, LHN4F) a new band appeared between 1550—1560 cm^{-1}, which may be assigned to asymmetric $\nu_{as}NO_2$ vibrations characteristic of aromatic nitro compounds. The band belonging to symmetric νNO_2 vibrations also appeared, although with lower intensity, at about 1340 cm^{-1}. This implies that simultaneously with oxidation, nitration also took place in the course of nitric acid treatment. Therefore, in this case regenerated humic acid may be considered as "nitrohumic acid".

Finally, in the IR spectra of all humic acid samples (especially lignite I and brown coal) the band at 1050 cm^{-1}, which is partly due to ν(Si—O—H) vibrations, points to very strong silicon and aluminium bonds due to the presence of humic acid-clay mineral complexes.

Functional groups

The results of IR spectroscopic investigations are also supported by analytical data of functional groups. Comparing the amount of acidic functional groups of humic acids (Table 7) with the corresponding values of initial raw coals (Table 7a), it can be established that the considerable increase in acidic OH in humic acids may be assigned to their carboxylic groups.

Based on IR spectroscopic investigations, the low number of carboxylic groups of raw coals can be interpreted mainly by the fact that the metal contaminations [Ca(II), Mg(II), Al(III) and Fe(III)] strongly bound in the carboxylate groups of raw coals could only be partly removed during the analysis of the functional groups.

Metal bonding to phenolic hydroxyls was found to be weaker. Therefore, the latter were accessible to Ba(II) ions during the analytical process even in the case of raw coals, which

Table 7a

Functional groups

	Total OH mequ./g		Carboxyl OH mequ./g		Phenolic OH mequ./g		Methoxyl mequ./g	
	coal	humic acid	coal	humic acid	coal	humic acid	coal	humic acid
Lignite I (Gyöngyösvisonta)	3.72	7.10	2.17	4.28	1.55	2.82	1.31	0.51
Lignite II (Cser-shaft)	4.78	8.24	1.06	4.62	3.72	3.62	0.63	0.22
Brown coal (Dudar)	3.70	6.34	0.96	3.62	2.74	2.72	0.70	0.00
Nitrated lignite II (Cser-shaft)	7.79	8.87	2.64	5.50	5.15	3.37	0.39	0.21

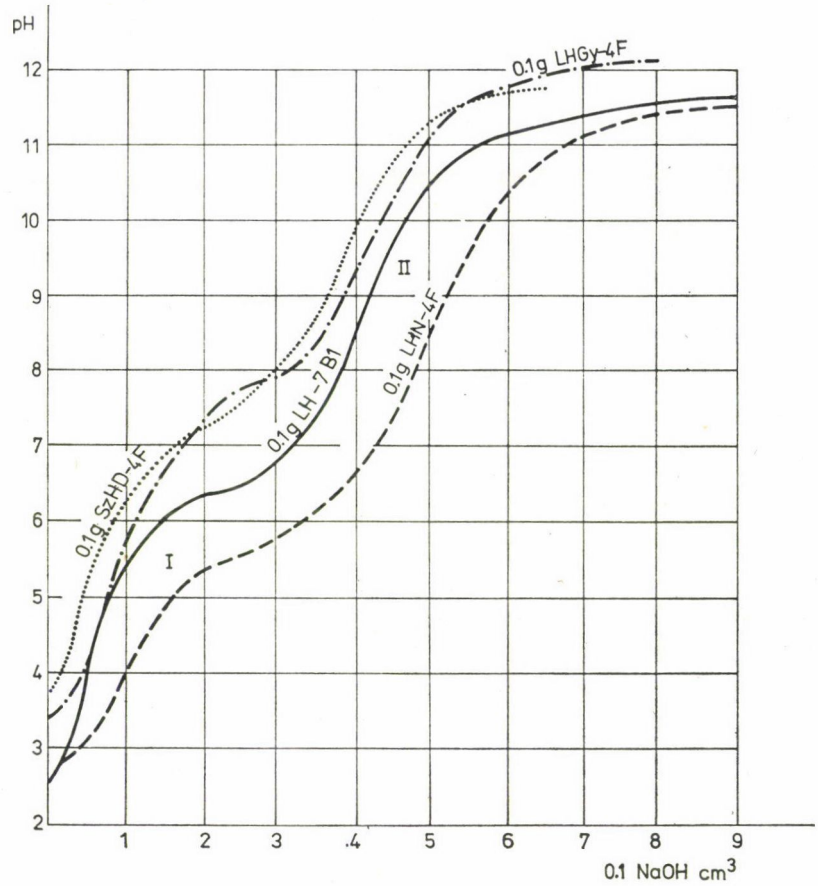

Fig. 7. Potentiometric titration curves of purified humic acids (see I and II in text)

explains the good agreement generally found between the phenolic OH values of raw coals and humic acids. The amount of methoxyl groups was lower in humic acids than in raw coals due to the high aliphatic hydrocarbon content of the latter.

The number of functional groups in humic acids unanimously decreases with coalification. The lower phenolic OH content of humic acid extracted from lignite I is, however, remarkable, and suggests a different chemical structure.

The distribution of oxygen functional groups in lignite II and nitrohumic acid was very similar, although in the latter an increase in the number of carboxylic groups and the total amount of acidity could be observed.

Acidimetric determinations

As is evident from the titration curves presented in Fig. 7, the titration periods of carboxylic (I) and phenolic hydroxyl groups (II) are well discernable in the case of humic acids extracted from lignite II, lignite I and nitrated lignite II. The titration curve of brown coal humic acid (SZHD4F) is, however, composed of 3 different sections: after the first section

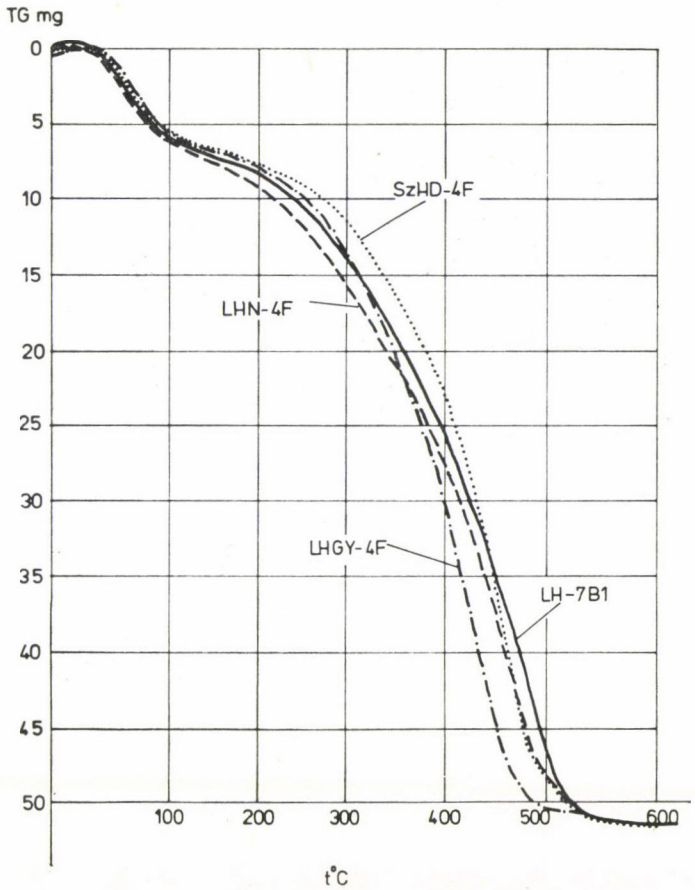

Fig. 8. TG curves of purified humic acid samples

indicating low carboxylic acid content (approx. 2.2 mequ/g), a transition can be observed between pH = 7.5—8.5, probably due to strong acidic phenolic hydroxyl groups. As a result of phenolic hydroxyl titration, a shift towards pH = 12 is shown in the curve.

Study of copper(II) ion bonding by complexometry

The metal ion bonding capacity of humic acids related to copper(II) ions has been determined by complexometry (MEISEL *et al.* 1979) at pH = 3.7. Comparison of the data obtained with those of total acidity values (Table 7) shows that copper(II) ion bonding is lower than could be expected on the basis of total acidity. The explanation of this phenomenon is that pH = 3.7 substitution of hydrogen ions by copper(II) ions is not complete, and in addition, a considerable amount of side ions (e.g. sodium ions) are bound to the humic acid polymer. Sodium ions were added to the solution in the course of pH adjustment. In our experience (LAKATOS *et al.* 1977b, SIPOS *et al.* 1978, VINKLER *et al.* 1976, MEISEL *et al.* 1979) and according to observations by KATCHALSKY *et al.* (1961) and KATCHALSKY (1964), such

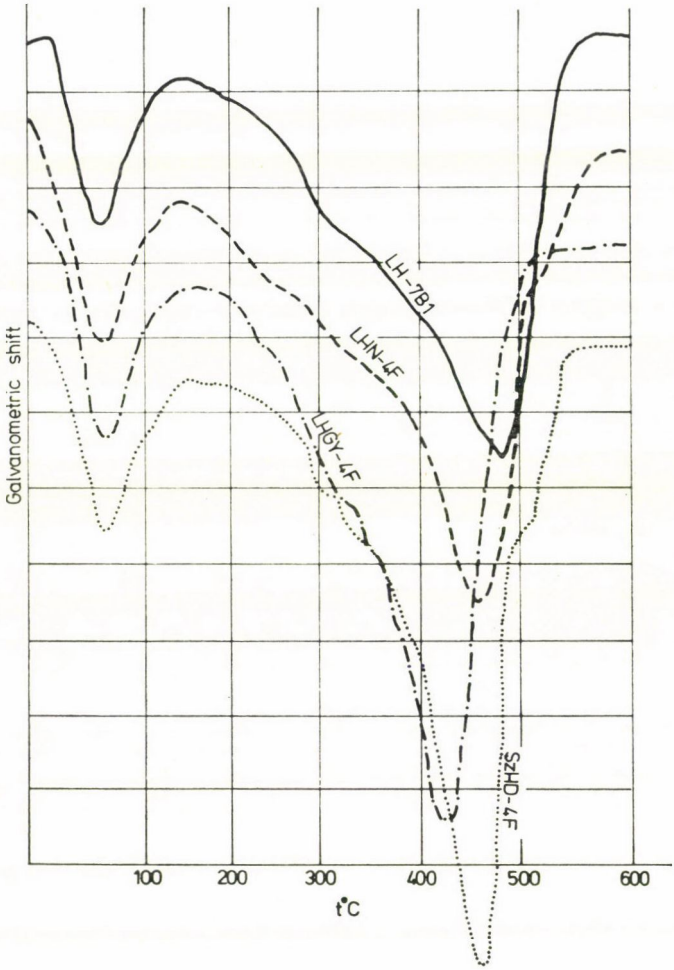

Fig. 9. DTG curves of purified humic acid samples
(abscissa): t°C
(ordinate): galvanometric shift

competition between different cations must also be taken into consideration in the interaction of polymers and metal ions.

The amount of copper(II) ions bound to humic acid molecules depends on the number and steric position of the carboxylic and phenolic hydroxyl groups. Since the number of oxygen functional groups decreases with the increase of coalification, the complexing ability of more coalified humic acid types may also be expected to decrease. This assumption is supported by our observations of copper(II) ion bonding to lignite II (3.40 mequ./g) and brown coal humic acids (1.50 mequ./g).

In spite of the high acid capacity of the nitrohumic acid of nitrated lignite II, only 2.25 mequ./g copper(II) bonding could be observed, due to the higher amount of sodium required for pH = 3.7 adjustment.

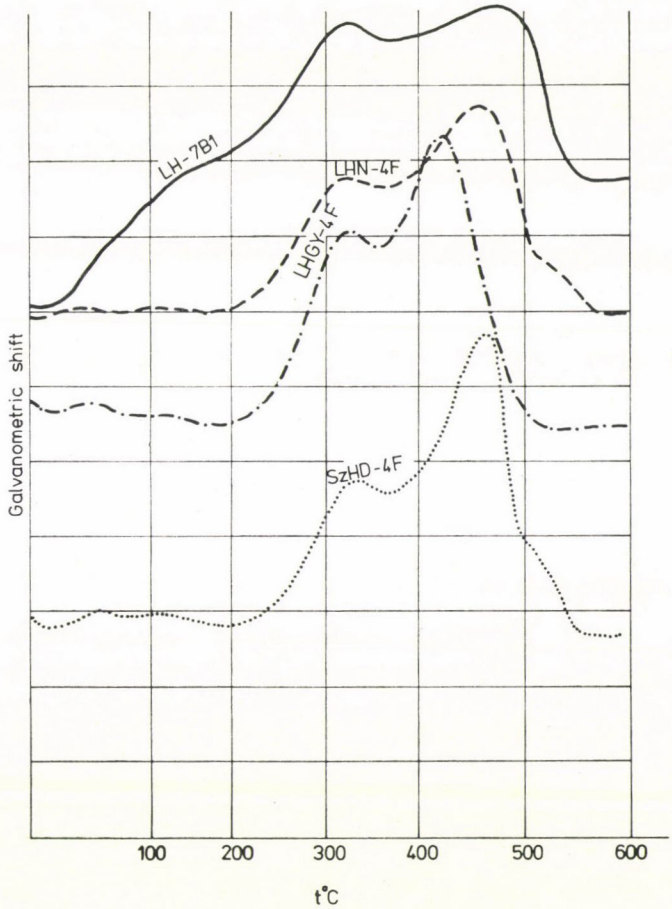

Fig. 10. DTA curves of purified humic acids
(abscissa): t°C
(ordinate): galvanometric shift

Thermal analysis

In the structure investigation of humic acids, thermal analysis has also been applied. Based on the method of JAMBU—DUPUIS (1975), metal contaminations were detected in the air stream.

Investigations were carried out for both raw (HS) and purified humic acids (THS) (Table 8). TG, DTG, and DTA curves are given only for purified humic acids (Figs 8—10).

DTA curves of high purity humic acids, plotted on the basis of data measured in the oxygen or air stream were very simple. At 70—80 °C a weak endotherm peak can be detected, at 300—320 °C there is a broad exotherm effect, and at 450—460°C a peak suggesting a strong exotherm process can be observed. The position of these peaks is much dependent on metal contaminations, which generally shift them towards lower temperatures.

The exotherm peak (1) may be assigned to the oxidation of the aliphatic chains of humic acids and peak (2) is due to the combustion of the aromatic core.

By calculating the percentage weight decrease in the temperature ranges 180—350 °C and 350—530 °C on the basis of TG curves, structural differences in the individual humic acid samples can be compared. As shown by the data in Table 8, the different degree of coalification in both raw (HS) and purified (THS) samples is evident. The weight loss of lignite humic acids, which contain substantial aliphatic chains was found to be 22—25% in the first range of temperature and 61—65% between 350—530 °C. In the case of brown coal, however, the ratio is shifted to 17% and 70%, respectively.

Table 8

Study of raw and purified humic acids by derivatographic methods

Initial raw coal type	Lignite I (Gyöngyös-visonta)		Lignite II (Cser-shaft)		Brown coal (Dudar)		Nitrated lignite II (Cser-shaft)	
	HS	THS	HS	THS	HS	THS	HS	THS
Ash (dry), %	7.37	2.68	1.27	0.66	5.45	1.54	1.45	0.88
Al 10^{-2}, %	98.2	29.2	16.9	7.45	22.8	8.17	15.3	5.08
Fe 10^{-2}, %	26.3	6.78	4.83	2.21	4.46	1.47	4.37	1.29
TG weight decrease, %								
180—350°C	22.0	25.0	23.0	21.0	18.0	16.0	24.0	23.0
350—530°C	61.0	62.0	61.0	65.0	68.0	71.0	60.0	61.0
DTG								
2nd min., °C	420 (520)	420 (520?)	475	480	440	460	460	460
DTA								
1st max., °C	300	325	316	330	320	325	300	325
2nd max., °C	420	470	470	480	440 (505)	460	440	470

Based on DTA maxima it may be observed that in the case of contaminated raw humic acid (HS) samples, exotherm peaks appear at somewhat lower temperatures than those of purified humic acid (THS). According to JAMBU—DUPUIS (1975) the maximum of the 3rd peak is decreased by trivalent metals bound to the carboxylate anion. The $Fe(OH)^{2+}$ ion complex of iron decomposes at 480 °C and its $Fe(OH)_2^+$ ion complex at 450 °C. The same applies to aluminium, with peaks appearing in the range of 480—510°C. The decomposition of calcium contaminations occurs at 520 °C.

The DTG curves of purified humic acids (THS) (Fig. 9) show a hardly discernible minimum at 520 °C for both lignite I and brown coal humic acids, which is due to the higher ash content of these samples and suggests the presence of calcium or aluminium contaminations. These minima at 520 °C were found to be more intensive in the case of raw humic acid samples (HS).

Metal humates

The structure of metal humates has been studied by various experimental methods (LAKATOS *et al.* 1977a, SIPOS *et al.* 1978, VINKLER *et al.* 1976, LAKATOS *et al.* 1977b, LAKATOS—MEISEL 1978, LAKATOS *et al.* 1977c).

By using purified humic acids, two salts (sodium, calcium) and two complexes, copper(II) and aluminium(III), have been prepared. The metal ions were chosen according to the following considerations: 1. increasing valency number, 2. complexing ability and 3. the effect of metal contaminations.

The salts were prepared by the reaction of humic acids with sodium hydroxide at pH = 12 and calcium hydroxide at pH = 9, respectively.

The IR absorption spectrum of sodium humate (NaLH7B1, Fig. 4B) revealed bands assigned to antisymmetric $\nu_{as}(COO^-Na^+)$ and symmetric $\nu(COO^-Na^+)$ vibrations at 1580 cm^{-1} and 1380 cm^{-1}, respectively. In the IR spectrum of calcium humate (CaLHB2, Fig. 5C) the band at 1590 cm^{-1} can be assigned to antisymmetric $\nu_{as}(COO^-Ca^{2+})$ vibrations.

For the preparation of the aluminium complex, an aqueous solution of lignite II humic acid (0.35%) at pH = 2.6 was mixed with a saturated aqueous aluminium chloride solution. The mixture was adjusted to pH = 3.5 to increase the number of bonded aluminium(III) ions. The pH value was not increased further, in order to avoid possible aluminium hydrolysis. The shoulder at 1700 cm^{-1} in the IR spectrum of aluminium humate (AlLH7B2, Fig. 5D) shows that at this low pH value, some hydrogens of the carboxylic groups were not completely substituted by aluminium ions. The band at 1615 cm^{-1}, instead of the usual position of the antisymmetric $\nu_{as}(COO^-Al^{3+})$ band at 1625—1630 cm^{-1}, points to the bonding of sodium ions.

In the IR spectrum of the copper humate sample, the low intensity band at 1710 cm^{-1} (CuLH8B1, Fig. 5E) indicates that at this higher pH value (pH = 5.5), not all hydrogen ions of the carboxylic groups were substituted by copper(II) ions. At higher pH values, however, hydrolysis of the copper(II) ion must also be taken into consideration. The antisymmetric $\nu(COO^-Cu^{2+})$ band at 1610 cm^{-1} was shifted to 1600 cm^{-1}, which shows that the sodium ions used for pH adjustment were also bound partially to carboxylate groups.

Copper(II) humate was also prepared from the pure humic acids obtained from nitrated lignite II. In the IR spectrum of copper(II) nitrohumate (CuLHN4, Fig. 2F), a very broad band can be observed at 1610 cm^{-1} compared to that of copper(II) humate prepared from raw lignite II humic acid. Therefore, considerable sodium ion bonding must be taken into account. The band at 1390 cm^{-1} contains symmetric $\nu(COO^-M^{+n})$ vibrations of copper(II) and sodium carboxylate. The structureless broad bands point to strong aggregation caused by copper(II) ions.

The analytical data of the copper content of copper(II) humates prepared from the four different kinds of humic acids are collected in Table 9.

Table 9

Experimental data of the copper humates prepared

Initial raw coal type	Lignite I (Gyöngyösvisonta)	Lignite II (Cser-shaft)	Brown coal (Dudar)	Nitrated lignite II (Cser-shaft)
Cu, %	32.3	17.8	29.1	12.6
Humic acid, % (calculated)	67.7	82.2	70.9	81.4
Cu atom	0.51	0.28	0.46	0.20
Cu, mequ./g HS	7.5	3.4	6,5	2.3
Cu, mequ./g HS (determined by complexometry)	1.75	3.4	1.5	2.25

The copper(II) mequ./g values for both lignite II humic acids and nitrohumic acids show good agreement with the analytical data determined by complexometry.

The copper content in lignite I and brown coal humic acids was, however, substantially higher, which cannot be interpreted by complex bonding. Very probably adsorption processes may also have participated in the bonding of copper(II) ions.

Molecular weight determination and study of aggregation with copper(II) ions

In the course of these investigations, the average molecular weights of humic acids were determined at appropriate pH values by analytical ultracentrifugation according to the sedimentation equilibrium method. Harmonic average molecular weight values have been

Table 10

Average molecular weight values of purified humic acids and Cu-humates

Initial raw coal type	Lignite I (Gyöngyös-visonta)	Lignite II (Cser-shaft)	Brown coal (Dudar)	Nitrated lignite II (Cser-shaft)
Humic acid:				
Ultracentrifugation $\overline{M}$ in Dalton	9,300	8,900	5,200	2,290
Harmonic average calculated on the basis of fractionation by gel filtration	7,447	9,200	4,226	1,509
Cu-humate:				
Ultracentrifugation $\overline{M}$ in Dalton	26,000	22,000	19,000	10,500
Harmonic average calculated on the basis of fractionation by gel filtration	24,138	19,960	9,042	7,360
Aggregation:				
Based on ultracentrifugation averages	2.79	2.27	3.67	4.62
Based on gel filtration harmonic averages	3.25	2.17	2.13	4.87

Table 10a

Changes in molecular weight with the degree of coalification

	O/C atomic ratio	$\overline{M}$ in Dalton (ultra-centrifugation)	Aggregation (based on $\overline{M}$ UC)	pH	Methoxyl, %	Al(III)	Fe(III)
						10^{-2}	
						%	%
Lignite I (Gyöngyösvisonta)	0.38	9300	2.79	3.4	0.51	29.20	6.78
Lignite II (Cser-shaft)	0.36	8900	2.47	2.6	0.22	7.45	2.21
Brown coal (Dudar)	0.31	5200	3.67	3.9	0.00	8.17	1.47
Nitrated lignite II (Cser-shaft)	0.42	2290	4.62	2.7	0.21	5.08	1.29

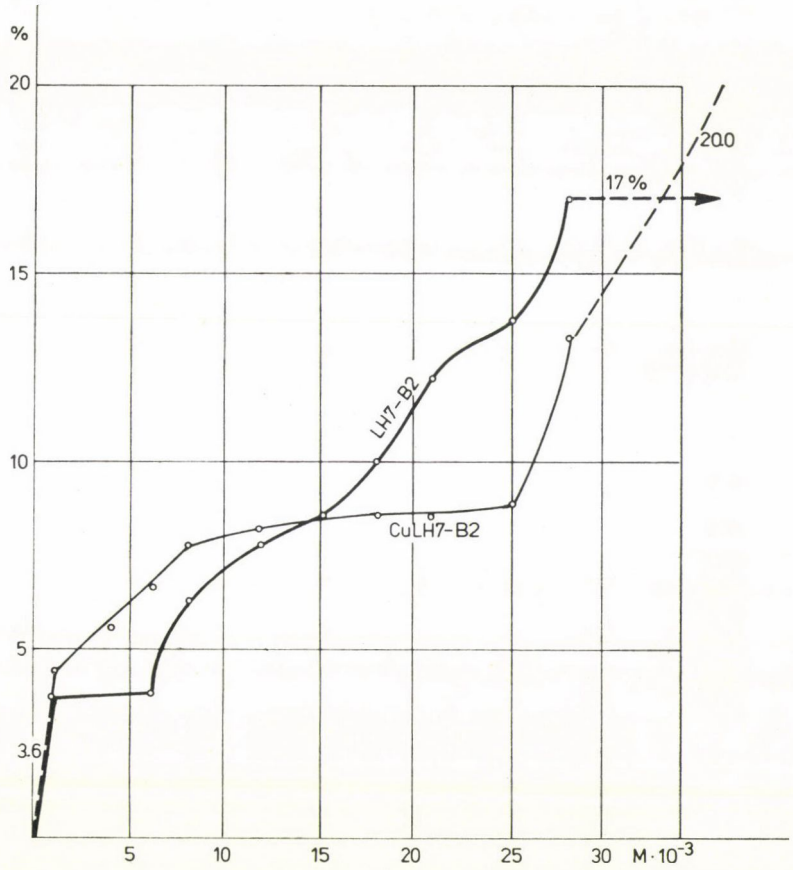

Fig. 11. Molecular weight distribution by gel filtration of purified humic acids extracted from lignite II, and Cu(II)-humate

calculated on the basis of molecular weight distribution after fractionation by gel filtration on a Sephadex G-50 dextrane at pH = 7.5. The degree of aggregation has also been studied during the formation of copper(II) humate. Average molecular weight values in Dalton (D) are summarized in Table 10 and molecular weight distribution curves are depicted in Figs. 11—14.

As is evident from Fig. 11, lignite II humic acid is an extremely heterodisperse substance. Due to the low molecular weight of the smallest fraction, the average molecular weight measured by ultracentrifugation was found to be 8900 D, contrary to the harmonic average value 9200 D calculated on the basis of fractionation by gel filtration.

The distribution curve of copper humate precipitated by copper(II) ions shows two distinct steps. The first at 8000 D and the second at 25 000 D. In the lowest four fractions (up to 8000 D) the curve shows a gradual increase. Approximately 24% of the substance falls into this molecular weight range, while the two highest fractions represent approx. 33% of the total amount of the substance. The intermediate fractions constitute the bulk of the material, approx. 43%. In these intermediate five fractions, the difference is altogether 1%. The average molecular weight value (22 000 D) measured by ultracentrifugation is in good

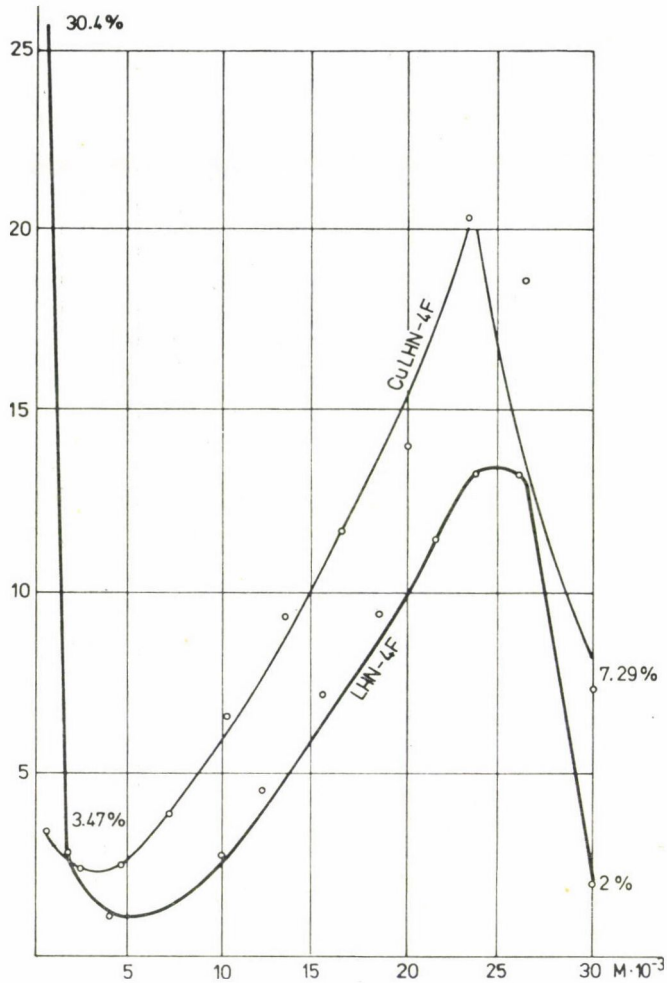

Fig. 12. Molecular weight distribution by gel filtration of purified humicacids extracted from nitrated lignite II, and Cu(II)-humate

agreement with the harmonic average molecular weight (19 960 D) calculated on the basis of the distribution curve.

Figure 12 presentse the molecular weight distribution curve of nitrohumic acid obtained from nitrated lignite II. A study of this curve shows that a substantial part of the material, approx. 56%, falls into the molecular weight range 15 000—30 000 D, but approx. 30% shows very low values (lower than 1000 D). This fraction, which is a mixture of degradation products, was formed in the course of nitric acid treatment. The harmonic molecular weight average calculated on the basis of fractionation is even lower (1509 D) than the average molecular weight of the sample measured by ultracentrifugation (2290 D), due to the presence of very low molecular weight fractions (30%).

It can be seen from Fig. 12 that the copper nitrohumate sample mainly contains higher molecular weight fractions. More than 70% of the substance is over 15 000 D. The average

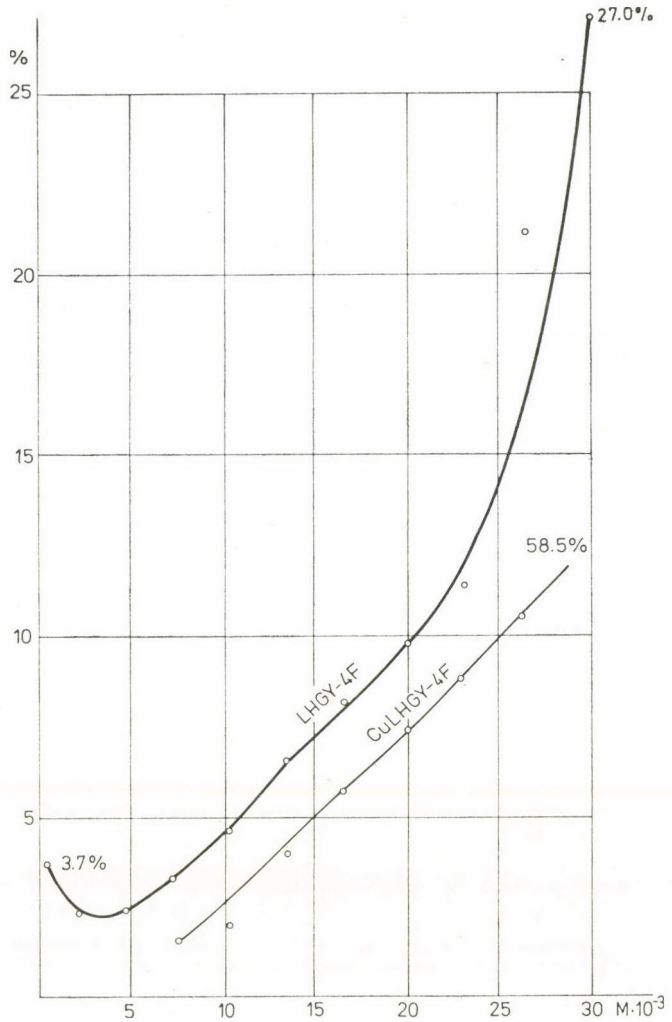

Fig. 13. Molecular weight distribution by gel filtration of purified humic acids extracted from lignite I, and Cu(II)-humate

molecular weight measured by ultracentrifugation is higher (10 500 D) than that obtained on the basis of the harmonic average value (7360 D). The molecular weight distribution curve shows that due to the effect of copper(II) ions the amount of the lowest fraction is reduced to a minimum (from 30% to 3%) while the ratio of high molecular weight fractions increased considerably.

Figure 13 gives molecular weight distribution curves for lignite I humic acids. The average molecular weight value of the humic acid sample measured by ultracentrifugation was the highest (9300 D), containing mainly high molecular weight fractions. According to the distribution curve, nearly 70% of the substance was above the molecular weight value 18 000 D and 27% fell into the highest molecular weight fraction. Accordingly, the value of the average

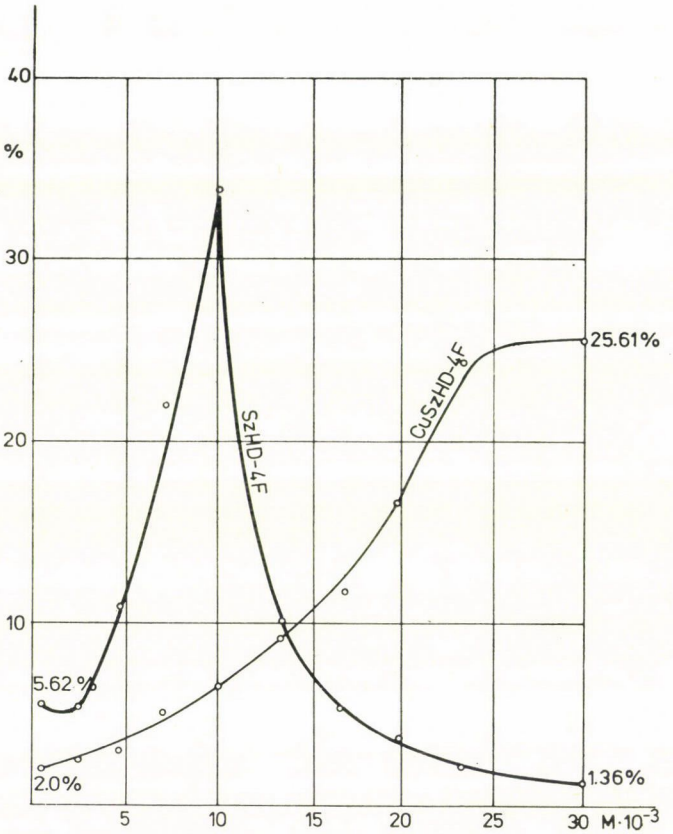

Fig. 14. Molecular weight distribution by gel filtration of purified humic acids extracted from brown coal (Dudar), and Cu(II)-humate

molecular weight obtained by ultracentrifugation is higher (9300 D) than the harmonic average (7447 D).

The distribution of the copper(II) humate precipitated by copper(II) ions shows a similar trend to that of the original humic acid sample. The amount of large fractions increased and the fraction above the value 28 000 D was 58.5%, but the average molecular weight was not higher here than 32 000—35 000 D. In this copper(II) humate sample molecular weight fractions were never below the value of 6000 D and the total amount of fractions lower than 12 000 D was not more than 3%. The average molecular weight value determined by ultracentrifugation was 26 000 D and the harmonic average was estimated at 24 188 D.

Figure 14 gives molecular weight distribution curves for humic acid (extracted from brown coal) and its copper(II) complexes. Coal humic acid produced molecular distribution curves with a rather sharp maximum at the intermediate fractions; the molecular weight distribution was relatively uniform, with approx. 45% in the range of 8500—15 000 D. The amount of the lowest and highest fractions was only 5.6 and 1.36%. The average molecular weight value determined by ultracentrifugation (5200 D) shows good agreement with the harmonic average (4226 D) calculated on the basis of molecular weight distribution.

The distribution of the copper(II) humate of brown coal humic acid shows a substantial change and is greatly shifted towards higher molecular weight fractions; 78% of the sub-

stances is above 15 000 D and 25.6% is above 25 000 D. The average value of 19 000 D determined by ultracentrifugation shows a substantial increase due to the effect of copper(II).

It may be established that the average molecular weight of natural humic acids decreases with the increase in the degree of coalification (Table 10). The reason for this phenomenon is presumably that humic acids from younger formations are richer in functional groups, the total acid number is higher and has lower pH values, thus more associated large polymers can form in aqueous solution.

The increase in the molecular weight due to copper(II) ions (see aggregation values in Table 10) shows a greater tendency for aggregation with the increase of coalification.

A certain anomaly can be observed between the molecular weight and pH values of lignite I humic acids. This contradiction suggests that in addition to coalification, the conditions of marsh formation also play a role in the development of the structure of humic acids. This difference in the structure of lignite I humic acid is also manifested by the following facts: higher percentage of aliphatic methoxyls, pointing to aliphatic side chains, lower phenolic hydroxyl content and the presence of some humic acid-clay mineral complexes due to considerable aluminium and silicon contaminations.

The molecular structure of nitro humic acid obtained from nitrated lignite II does not fit into the order found for the degree of coalification of natural humic acids where, due to degradation, molecular weight is only as high as 2290 D in spite of the low pH value. The highest degree of aggregation due to the effect of copper(II) ions can be observed in this case.

In the course of our investigations an attempt was made to point out differences between the molecular structure of natural and regenerated humic acids, which may lead to a better interpretation of the differences observed in the utilization of humic acid substances and preparations in soil improvement and plant cultivation experiments.

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Prepared at the Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Mining Research Institute, Budapest, Colloid Chemical Institute, József Attila University, Szeged.

B. Lakatos, J. Korbuly, J. Meisel, S. Sipos

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THE RHIZOSPHERE EFFECT OF SOME FRUIT TREES ON PHYSIOLOGICAL GROUPS OF SOIL MICROBES

According to the results of investigations carried out over the years, bacteria and actinomycetes have a great role in producing specific replant disease in apple, but they are not known pathogens. Some people consider that a species of *Actinomycetales* attacks the roots of apple. Others hold, on the basis of a number of experiments, that *Pseudomonas fluorescens* is responsible. Apple replant disease could be stopped with a dose (1500 ppm) of Agrimycin in the soil (BUNT—MULDER 1973).

In a monoculture, with a predominance of specific root exudates and plant residues, a one-sided, specific microflora develops. For example, the root exudates of pea and oat stimulate the organisms from the rhizosphere more than those from outside this zone. The selective stimulation of short Gram-negative rods appears to be one of the best established criteria for rhizosphere effect (ROVIRA 1956).

There are antibacterial substances in some plants. It has been established that Gram-positive bacteria are sensitive to aqueous extracts of dry residues from certain higher Egyptian plants (ZAYED *et al.* 1971).

By the third day after the planting of wheat seeds, there were more than twice as many bacteria in the rhizospheric soil as in the control soil. Qualitative differences were also apparent at this time in that methylene blue reducing bacteria, ammonifiers, denitrifiers, gelatin-liquefying and starch-hydrolysing types were preferentially stimulated (RUATT 1959).

According to experiments on many plants (*Gramineae*, *Cruciferae*, *Polygonaceae*, *Umbelliferae*, etc.) the number of aerobic, non-sporulating bacteria was greater, while that of sporulating and facultative anaerobic bacteria was diminished in the rhizosphere; obligate

anaerobes (e.g. *Clostridium* sp.) are not generally found. Often the quantity of actinomycetes diminishes too (KAUNAT 1963).

The root exudates of maize were explicitly toxic in higher concentrations to *Nitrosomonas* sp. (MOLINA—ROVIRA 1964).

STILLE (1958) reports on the behaviour of *Azotobacter* in the rhizosphere. For the *Phaseolus*, *Beta*, *Cucurbita*, *Brassica*, *Allium*, *Solanum* and *Pisum* genera the R : S ratio was below zero, i.e. the *Azotobacter* was repressed. He also found that near the roots the non-sporulating, Gram-negative microbes are stimulated, e.g. *Pseudomonas* and *Xanthomonas*, but chiefly *Pseudoudomonas fluorescens*, which kills by toxin and consumes the cells of *Azotobacter*.

On the other hand the root exudates of pea stimulated *Pseudomonas fluorescens*, *Azotobacter chroococcum*, etc. MARATHE—RANGSWAMI 1973). According to PÁNTOS—PÁNTOS (1959) winter barley, maize and fallow are favourable to the propagation of *Azotobacter* sp.

The "irrigation soil sickness" is similar to specific soil sickness (= specific replant disease), but it is not so specific. The number of bacteria and actinomycetes increases, but the quantity of fungi decreases (HIRTE 1961).

A marked rhizosphere effect was observed on fluorescent pseudomonads in apple, where a maximum R : S ratio of 131 was recorded (BARCLAY—CROSSE 1974).

PICCI *et al.* (1971) investigated the genera *Pseudomonas*, *Bacillus*, *Micrococcus*, *Proteus*, *Sarcina*, *Achromobacter* and *Escherichia* on extracts from peach root which proved to be toxic to lettuce. It was established that none of these microbes is toxin-lysing, but they multiply well in these substances.

The microflora of replanted and diseased peach roots was examined by LEPIDI *et al.* (1974). The results showed that the populations of healthy roots and of diseased ones differ considerably. The latter were more active and they metabolized the different substances better, compared to the organic acids.

"Katznelson observed a rhizosphere effect of mangels on gas-producing anaerobes, very closely related to, if not identical with, *Clostridium pasteurianum*. Krassilnikov and Vozniakovskaya noted the accumulation of *Clostridium pasteurianum* in the rhizosphere of wheat and corn" (KATZNELSON—STRZELCZYK 1961).

In our experiments the microorganisms from the root surface, from the rhizosphere of some stone fruit trees and from outside this zone were investigated. First the quantitative differences were examined, then, based on this information, microbiological data characterizing the replant disease were studied.

Samples

Three soil samples were taken from each of two rhizospheric zones (depth: 5—25 cm). These zones were 0.5 and 2 m, respectively, from the trunks of apricot and sour cherry trees. Samples were also taken from root-free soils. The root pieces (diameter: 1—4 mm) were investigated separately. All samples are examined immediately.

In spring some fruit seedlings (apricot, apple, sour cherry and peach) and barley were planted in "good" arable soil, in pots. A pot served as irrigated fallow in this experiment. In autumn the effect of the roots was investigated by counting the anaerobic nitrogen-fixing and denitrifying microbes from dried soil samples.

In addition, some soil samples from fruit trees (apple, peach, apricot and sour cherry) and wheat were examined in a similar manner.

Counting methods

1. Dilution tubes. The microbes of the various physiological groups were counted by the most probable number (MPN) technique using 10-fold dilutions, taking four replicate 1 ml aliquots from each dilution (PARKINSON *et al.* 1971, HORVÁTH 1974). In positive tubes the microbes multiplied. The groups investigated were as follows: cellulose-decomposing, ammonifying, nitrifying (from ammonia), denitrifying, urea, pectin and glucose-fermenting (butyric

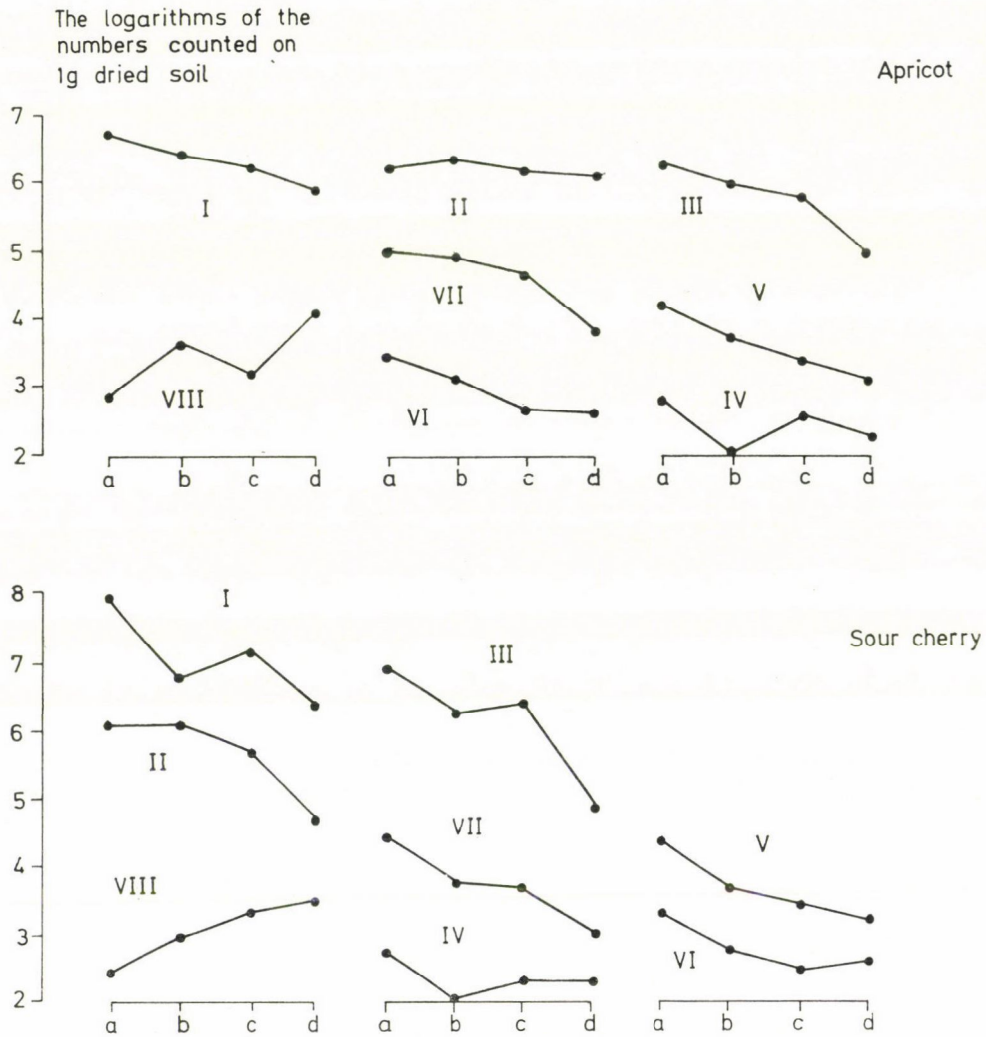

Fig. 1. The quantity of microbes isolated from fresh soil samples. Physiological groups (averages of three repetitions; MPN method), (a = root surface, b = 0.5 m from trunk, c = 2 m from trunk, d = 6 m from trunk, I = pectin fermenting microbes, II = ammonifying microbes, III = denitrifying microbes, IV = nitrifying microbes, V = urea fermenting microbes, VI = cellulose decomposing microbes, VII = glucose fermenting microbes, VIII = nitrogen-fixing microbes)

acid forming) microbes. The selective culture media were made according to POCHON's (1954) manual.

In experiments concerning the anaerobic microbes *(Clostridium)* the anaerobic incubation condition was assured by a paraffin layer on the surface of liquid culture media in tubes. (Incubation in N-gas gave similar results.)

2. Agar plates (Pour plate technique). 1 ml of diluted soil suspension was mixed on a plate with 10 ml of the medium for denitrifying bacteria.

3. Surface inoculated plates. Aliquot samples of dilutions were pipetted onto the surface of nitrogen-deficient silica gel plates for counting *Azotobacter*.

The data obtained are presented in the tables. These demonstrate a fact which has often been proved: in general the cell number of microbes on the root surface and in the rhizosphere is larger than in the soil outside this zone; R : S (rhizosphere soil ratio) > 1. This was true for the physiological groups, but there was an exception: the group of nitrogen-fixing microbes (Fig. 1). The cell number decreased in the rhizosphere, and particularly on the root surface.

Table 1

The numbers of Azotobacter (A) and denitrifying (D) microbes from fresh soil samples (Counted on 1 g dried soil; averages of 6 plates)

Soil samples from	A$\times 10^3$	D$\times 10^5$	A : D, %
Ploughed land, wheat	5.2	12	0.4
Apricot rhizosphere	12.0	20	0.6
S. cherry rhizosphere	9.0	20	0.5
Apple rhizosphere	4.7	12	0.4
Peach rhizosphere	1.6	24	0.06

Table 2

The numbers of nitrogen-fixing (NF) and denitrifying (D) microbes isolated from fresh soil samples (Counted on 1 g dried soil; MPN technique)

Soil samples from	NF$\times 10^3$	n	D$\times 10^5$	n	NF : D, %
Ploughed land, wheat	10.8 ± 3.4	12	2.4 ± 2.1	9	4.5
Apricot rhizosphere	4.0 ± 0.8	9	5.1 ± 1.1	6	0.8
S. cherry rhizosphere	0.9 ± 0.9	6	2.5 ± 0.8*	3	0.4
Apple rhizosphere	6.6 ± 1.6	6	2.8 ± 0.9*	3	0.4
Peach rhizosphere	14.2 ± 4.2	9	29.8 ± 9.2	5	0.5
Apple seedling root surface	2.0 ± 0.1	12	7.4 ± 1.5	5	0.3
Peach seedling root surface	4.3 ± 1.0	15	30.4 ± 5.3	5	0.1

P = 5%, exc. * = 10%.

The most significant genus of free-living nitrogen-fixing bacteria is *Azotobacter*. The dilution tube culture method is more favourable for anaerobic organisms. Therefore some rhizosphere soil samples (apricot, sour cherry, apple, peach and wheat) were examined on Winogradsky-silica gel. Parallel to this, denitrifying microbes were counted by the pour plate technique. According to the results (Table 1), Azotobacter was not repressed except in the peach soil samples.

On the other hand, in the dilution tube culture the quantity of nitrogen-fixing and glucose-fermenting microbes characterized by gas and butyric acid production (chiefly *Clostrigium* sp.) was reduced in relation to the denitrifying microbes representing the total number of microorganisms (Table 2).

Cultures inoculated from dried soil samples in nitrogen deficient media and incubated under anaerobic conditions have given similar data, compared to the number of denitrifying microbes (Table 3).

Table 3

Proportion of anaerobic nitrogen-fixing microbes as a percentage of denitrifying microbes (Isolation from dried soil samples, MPN technique, 4 repetitions)

Wheat rhizosphere	5.9
S. cherry rhizosphere	1.6
Apricot rhizosphere	1.0
$LSD_{1\%}$	3.4

Table 4

Proportion of anaerobic nitrogen-fixing microbes expressed as a percentage of the denitrifying microbes isolated and of barley planted in a standard soil (MPN technique, 4 repetitions)

Barley rhizosphere	7.1
Apricot rhizosphere	3.9
Peach rhizosphere	6.8
Sour cherry rhizosphere	3.7
Apple rhizosphere	4.2
"Irrigated fallow"	2.7
$LSD_{5\%}$	3.1

The pot experiments also gave similar results: in the rhizosphere of apricot and sour cherry the relative quantity of anaerobic nitrogen-fixing microbes was low, as it was in the soil of the "irrigated fallow", but in the rhizosphere of peach and apple (all seedlings), and particularly of barley, it was high.

The following conclusions may be drawn from the results of the experiments: 1. The rhizospheric effect of stone-fruit trees (apricot, sour cherry, peach) is characterized by a decrease in the nitrogen-fixing microbes. 2. *Azotobacter* sp. was repressed only in the soil of old

peach trees. 3. Anaerobic nitrogen-fixing microbes (*Clostridium* sp.) were reduced in the rhizosphere of apricot and sour cherry trees and seedlings. 4. The number of anaerobic nitrogen-fixing microbes (*Clostridium* sp.) was relatively high in the soil of wheat and barley.

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Prepared at the Fruit Research Station, Cegléd

G. D. Kovács

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CHANGES IN THE AGRONOMIC PROPERTIES OF MAIZE (ZEA MAYS L.) HYBRIDS WITH DIFFERENT GENOTYPES AS A RESPONSE TO EVEN AND UNEVEN SPACING

In commercial crop production in Hungary farm plots are often encountered where the evenness of spacing is unsatisfactory. The spacing between 200 plants per ha in each of thirty maize fields sown with the same type of corn planter was measured at the 6—8 leaf

stage. The relative standard deviation (s%) of the plant spacing ranged between 45.0 and 91.0%.

There are very few literary data on the effect of uneven plant spacing. In the case of sunflower REMUSSI *et al.* (1974) demonstrated an 11.7 to 28.9% reduction in yield depending on the degree of unevenness. Farm experience led GYŐRFFY (1976) to call attention to the importance of uniform spacing. MOCK—HEGHIN (1976) studied the effect of row and random sowing patterns in two types of stands for two hybrids, and established that both hybrids and both stands gave better yield results when sown in rows with uniform spacing. PINTÉR *et al.* (1978) established that the yields of hybrids with different genotypes show different trends as a result of uneven spacing.

As a continuation of this work a two-year experiment was carried out to investigate the relationship between uneven spacing and grain yield, as well as between the yield components and parameters indicating growth and development.

The material used in the investigations consisted of two commercially grown hybrids produced at the Cereal Research Institute. The hybrids, FAO 360 and FAO 565, commercially available under the names K SC 360 (A90×153R) and Sze SC 565 (Oh43/K×A632), respectively, had practically identical optimum plant numbers but different genotypes. In the results of small plot experiments presented in the paper of PINTÉR *et al.* (1978) the latter hybrid is mentioned as a A632×A619 based hybrid, since it is a combination with similar genetic, morphological and agronomical properties.

The experiments were carried out in both years with the same method, at the same place, at the institute's "Ságvári" station in Szeged. The treatments are shown in Table 1.

The experiment was laid out in a random block design, with three replications. Each plot consisted of 10 plants sown in a single row. Both hybrids were sown with a sowing gun to give an almost identical density in both years, and by thinning at the 6—8-leaf stage stands of even and uneven plant distribution were obtained. The distance between the rows was 70 cm in both years. Ignoring technical errors, the plant stand was 5.0 plants/m² in 1976 and

Table 1

Plant spacings and their standard deviation in the treatments

Factors examined	A90×153R		Oh43/K×A632	
	even	uneven	even	uneven
1976				
— plant spacing, cm	28.65	32.63	28.60	30.13
— standard deviation of plant spacing, cm	2.96	24.39	3.10	24.32
— F-value of standard deviation	67.97		40.86	
— $P_{0.01}$%	2.64		2.64	
1977				
— plant spacing, cm	23.05	22.95	23.45	24.83
— standard deviation of plant spacing, cm	2.66	19.03	2.76	19.31
— F-value of standard deviation	51.20		48.68	
— $P_{0.01}$%	2.64		2.64	

6.0 plants/m² in 1977. The average values for plant spacing showed no significant differences between the treatments in either of the years. The unevenness of the plant distance was exactly the same in the small plots, that is, the distance between plants in the same position (e.g. between the 5th and 6th plant of the plot) was identical. The spacing of the plants which served as a basis for calculating the values in Table 1 was measured after thinning, at the 8—10-leaf stage.

In determining the degree of unevenness the farm data mentioned in the introduction were taken into consideration, so the relative standard deviation (s%) of plant spacing in the uneven treatments ranged from 74.74 to 82.91%.

To eliminate the border effect 14 plants were sown in each plot, and the first and last ones were not taken into account in the evaluation. To eliminate the neighbour effect a row of the same hybrid was sown on each side of the plot with uniform plant distribution.

The processing of data collected during the vegetation period, as well as harvesting and evaluation were carried out for each plant individually. Harvesting was carried out at a 30% grain water content, and since the hybrids have different vegetation periods, harvesting took place at different dates. The ear crop was dried to a 14% grain water content before processing.

The uniform soil quality of the experimental plots, the nutrient level of the soil, and the amount and distribution of rainfall during the vegetation period did not disturb the manifestation of the treatment effects. The data were evaluated by analysis of variance.

Table 2

Grain yields per plant for hybrids with different genotype, and trends of factors influencing the yield in the case of even and uneven spacing

Factors examined		A90×153R		Oh43/K×A632	
		even	uneven	even	uneven
Air-dry (14% H_2O) grain yield (number of grains per plant)	1976	181.20	191.6	224.90	185.50
	$SD_{5\%}$	ns		13.60	
	1977	159.73	163.05	202.03	184.30
	$SD_{5\%}$	ns		17.00	
Number of ears per plant	1976	1.00	1.00	1.00	1.00
	$SD_{5\%}$	ns		ns	
	1977	1.00	1.00	1.00	1.00
	$SD_{5\%}$	ns		ns	
Length of pollinated ears (cm)	1976	17.20	18.00	19.70	17.90
	$SD_{5\%}$	ns		1.08	
	1977	18.00	17.25	17.65	15.58
	$SD_{5\%}$	ns		1.39	
Number of grain rows	1976	13.53	13.73	17.33	17.53
	$SD_{5\%}$	ns		ns	
	1977	12.70	13.00	17.50	17.10
	$SD_{5\%}$	ns		ns	
Thousand-grain-weight, g	1976	345.90	353.30	306.50	298.70
	$SD_{5\%}$	ns		ns	
	1977	341.30	333.70	303.60	306.40
	$SD_{5\%}$	ns		ns	

ns = No significant difference.

The main aim of our investigations was to find out what trends would be shown by the yield results of hybrids with different genotypes and the factors influencing the yield as a response to uneven spacing. The results are contained in Table 2.

In 1976 the average grain yield per plant was higher in all four treatments than in 1977. This did not, however, disturb the tendencies indicating treatment effects.

A significant difference between the two treatments in dry grain yield per plant was only found in the Oh43/K×A632 combination in both years. With A90×153R no significant difference was obtained in either of the years. It was remarkable that while in the former hybrid the uneven treatment gave the lower yield, in the latter the lower values were obtained in both years with the even distribution of plants (though the differences were not significant), which suggests that this hybrid probably tolerates a higher degree of uneven spacing without any loss of yield.

The data unequivocally confirm our earlier statement (PINTÉR *et al.* 1978) that the yield results for hybrids with different genotypes do not show the same trend under the influence of uneven spacing. There are genotypes (Oh43/K×A632 in our experiment) which show a significant yield reduction as a response to uneven sowing, while in others (A90×153R in our experiment) the yield remains unchanged.

Parallel to the grain yield, changes in the yield components were also examined, as shown in Table 2. In the hybrid A90×153R, similarly to the result obtained for the grain yield, none of the factors examined were found to be significantly different in the two treatments. In the case of Oh43/K×A632, where the grain yield showed a significant decrease in both years under the influence of uneven spacing, significant differences with a trend similar to that of the former hybrid were only obtained in both years for the length of the pollinated ear.

This suggests that the yield decrease observed as a result of uneven spacing is manifest primarily in the reduced length of the ear.

Besides the grain yield per plant and the trends of the yield components the growth and development of the plants was also followed during the growth season. Development was characterized by the number of days from emergence to blossoming of male and female flowers, while growth was characterized by the height of the fully developed plant. The data are shown in Table 3.

Table 3

Number of days from emergence to flowering, and heights of fully developed plants as a function of even and uneven spacing

Factors examined		A90×153R		Oh43/K×A632	
		even	uneven	even	uneven
Number of days from emergence to male flowering	1976	66.30	66.90	71.80	72.80
	$SD_{5\%}$	ns		0.78	
	1977	62.20	62.26	68.36	69.70
	$SD_{5\%}$	ns		1.28	
Number of days from emergence to female flowering	1976	66.10	66.60	72.40	73.40
	$SD_{5\%}$	ns		0.76	
	1977	62.76	62.60	70.20	71.40
	$SD_{5\%}$	ns		1.02	
Height of fully developed plant, cm	1976	233.20	233.30	250.20	253.0
	$SD_{5\%}$	ns		ns	
	1977	231.50	234.50	260.30	263.50
	$SD_{5\%}$	ns		ns	

ns = No significant difference.

In the case of A90×153R there was no difference between the two treatments in any of the parameters indicative of development, while in the hybrid Oh43/K×A632 both the male and female flowers blossomed significantly later in the uneven stand than with a uniform distribution of plants.

As for the height of the fully developed plants, no significant differences were found in the two years between the two treatments in either hybrid.

The data in Table 3 show that the negative effect observed for the grain yield of the hybrid sensitive to uneven plant distribution is manifest in its development as well. On the other hand, this tendency was not observed in its growth.

In order to decide what properties determine the response of hybrids to uneven spacing, investigations more extensive than those described in this paper are needed.

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Prepared at the Cereal Research Institute, Szeged

L. PINTÉR, J. NÉMETH, Z. PINTÉR

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RESEARCH ON HYBRID WHEAT AT MARTONVÁSÁR. VI

Experiments with hybrid wheat at Martonvásár have been reported on in several papers over the last ten years (RAJKI—RAJKI 1966, 1967, 1968, 1970, RAJKI *et al.* 1976). The results of flowering biology studies on wheat at Martonvásár, a knowledge of which promotes more reliable seed production in hybrid wheat, have also been published (CSÁSZÁR—RAJKI 1975a, b, c, 1976, 1977). In this paper the results of small plot experiments carried out in 1973—1976 with combinations of F_1 plants obtained from open pollinated cytoplasmic male sterile×restorer crosses are presented.

The hybrid seed used in the experiments was produced at Martonvásár (CSÁSZÁR—RAJKI 1977). Separate trials were sown for F_1 hybrids produced with the following restorers:

1. Rf Mironovskaya 808 (hereafter: Rf Mir.808).*

2. Rf (ms Mironovskaya 808×Prof. Marchal), hereafter: Rf (ms Mir.808×Prof. Marchal),

3. Primepi.

* The restorer Rf Mir.808 was produced by using Wilson's fertility restorer.

Besides the F_1 hybrids each trial included the fertile initial forms of the male sterile analogues used in producing the hybrid seed: Kavkaz, Avrora, Skorospelka 35, Bezostaya 1, Krasnodar 1, Jubileinaya 50, as well as the wheat varieties Mironovskaya 808 and Fertődi 293, and 2 prospective Martonvásár varieties. The restorers used to produce the hybrids were also sown in the trials. Each trial thus included a total of 42 factors.

Sowing took place in the trial grounds of the Institute at Martonvásár, on 26 September in 1972, on 2 October in 1973, on 19 October in 1974 and on 22 October in 1975. The trials were set up in four replications, in a random block design. The seed was sown at a rate of 80 per plot with a hand seeder in 1×0.4 m plots with 10×5 cm spacing. At the shorter sides of the plots 50 cm paths were left, while at the longer sides neither path nor row was left between the plots. The first and last experimental plots in the column were bordered by a four-row plot of Bezostaya 1.

The plots were fertilized in autumn with a 1 : 1 : 1 mixture of 200 kg/ha NPK active agent. The plant height including the length of spike was measured in the field. The grain yield of each plot was weighed separately after harvesting and threshing. The thousand-grain-weight was determined by weighing 3×500 grains.

The winters in 1972—1975 were not severe enough to enable frost resistance and winter hardiness to be evaluated in the field.

In the present phase of our hybrid wheat experiments one of the most important tasks is to compare the yields of F_1 hybrids with those of varieties included in commercial production at present or in recent years. In our experiments the largest yield, which was taken as 100% in comparing the F_1 hybrids, was produced by a different variety each year. There was no significant difference in yield between the standards and the other varieties included in the experiment, but as far as the trend was concerned Martonvásári 3 was found to be the best wheat variety in 1972—73, Fertődi 293 in 1973—74, Skorospelka 35 in 1974—75 and Jubileinaya 50 in 1975—76. Further, it can be established that none of the varieties consistently tended to give the largest yield every year.

The yield data of the most productive F_1 hybrids are shown in Table 1. F_1 hybrids were produced with the restorer Rf Mir.808 for four years, and of these the combination ms Bezostaya $1 \times$ Rf Mir.808 was found to be the best in the first three years of the experiment, while in the fourth year the F_1 hybrid of ms Kavkaz $\times$ Rf Mir.808 gave the largest yield. The yield of F_1 combinations produced using Rf Mir.808 as the restorer exceeded the yield average of the best varieties by 19.5% on the average of the years 1972—1976.

Table 1

Yield of F_1 hybrids compared to that of the highest yielding variety (%)

Year	♂	Rf Mir. 808		Rf (ms Mir. 808 × Prof. Marchal)		Primepi	
1972—1973	♀	ms Bez. 1	128.0*	—	—	ms Skor. 35	115.9
1973—1974		ms Bez. 1	115.1	ms Avrora	119.8*	ms Jub. 50	116.8
1974—1975		ms Bez. 1	124.0*	ms Kavkaz	135.7**	ms Jub. 50	100.3
1975—1976		ms Kavkaz	111.0	ms Bez. 1	135.3**	—	—

* The difference between F_1 and the highest yielding variety is significant at the 5% level.

** The difference between F_1 and the highest yielding variety is significant at the 1% level.

Investigations on F_1 hybrids produced with the restorer Rf (ms Mir.808 × Prof. Marchal) were not begun until 1973—74, as the restorer was not ready previously. That year ms Avrora × Rf (ms Mir.808 × Prof. Marchal) proved to be the best combination, outyielding the highest yielding variety by 19.0%; in 1974—75 ms Kavkaz × Rf (ms Mir.808 × Prof. Marchal) produced 135.7%, and in 1975—76 ms Bezostaya 1 × Rf (ms Mir.800 × Prof. Marchal) 135.3% compared to the most productive variety. On the average of the three experimental years the surplus yield of the F_1 hybrids was 30.3% compared to the average yield of the standards during this period.

Of the F_1 hybrids produced with Primepi as restorer the combination ms Skorospelka 35 × Primepi produced the largest yield in the first year of the experiment. In the subsequent two years the combination ms Jubileinaya 50 × Primepi proved to be the best. However, neither of these combinations produced the 20% or so yield surplus necessary if hybrid wheat production is to be profitable. The F_1 hybrids produced with Primepi as restorer gave an 11.0% larger yield than the average yield of the best varieties, averaged over three years. The study of F_1 hybrids produced with the restorer Primepi has therefore been discontinued.

When comparing the three year yield averages of F_1 hybrids produced with different restorers it is found that those produced with the pollen donor Rf (ms Mir.808 × Prof. Marchal) gave the highest yield, while F_1 hybrids produced with Primepi as restorer gave the lowest yield. The data in Table 1 show that the yields of F_1 hybrids produced with the restorers Rf Mir.808 and Rf (ms Mir.808 × Prof. Marchal) from the male sterile analogues of Bezostaya 1 and Kavkaz merit closer examination (Table 2).

Table 2

Yield of F_1 combinations of Bezostaya 1 and Kavkaz compared to the highest yielding variety (%)

Year	♂	Rf Mironovskaya 808			
1972—1973	♀	ms Bezostaya 1	128.0*		
1973—1974		ms Bezostaya 1	115.1	ms Kavkaz	102.2
1974—1975		ms Bezostaya 1	124.0*	ms Kavkaz	115.5
1975—1976		ms Bezostaya 1	103.1	ms Kavkaz	111.0
Mean			117.5		109.6
Year	♂	Rf (ms Mir. 808 × Prof. Marchal)			
1972—1973	♀				
1973—1974		ms Bezostaya 1	94.8	ms Kavkaz	112.5
1974—1975		ms Bezostaya 1	118.5	ms Kavkaz	135.7**
1975—1976		ms Bezostaya 1	135.3**	ms Kavkaz	128.4*
Mean			116.2		125.5

* The difference between F_1 and the highest yielding variety is significant at the 5% level.

** The difference between F_1 and the highest yielding variety is significant at the 1% level.

The data from three years of examinations given in Table 2 show that with the restorer Rf Mir.808 the highest yield was given by the F_1 hybrid of ms Bezostaya 1, while if Rf (ms Mir.808 × Prof. Marchal) was used as restorer the F_1 hybrid of ms Kavkaz gave the highest yield. From the point of view of hybrid wheat production the F_1 hybrid obtained by crossing ms Kavkaz with the Rf (ms Mir.808 × Prof. Marchal) restorer seems to be promising, since the three year yield average of the combination was 25.5% higher than the yield averages of the varieties which gave the largest yields in the individual years.

The yields of the restorers used in producing the F_1 hybrids are also worth studying (Table 3). The yields of the restorers, similarly to the data of Table 1 and 2, are expressed as a percentage of the yield produced by the highest yielding variety.

Table 3

Yields of restorers compared to the yield of the highest yielding variety included in the experiment
(%)

Year	Rf Mir. 808	Rf (ms Mir. 808 × Prof. Marchal)	Primepi
1972—1973	95.4	—	55.9
1973—1974	81.2	60.0	57.5
1974—1975	83.2	100.0	68.6
1975—1976	79.7	85.6	—
Mean	84.9	81.9	60.6

Of the restorers examined Rf Mir.808 gave the largest yield, and this restorer also showed the lowest yield fluctuation. Rf (ms Mir.808 × Prof. Marchal) yielded only 60% as much as the standard in 1974, but it gave a yield identical to that of the standard in the following year, and a yield which was about the average of the two previous years in 1976. The three year yield average of the restorer approached that of the variety Bezostaya 1. However, owing to the great fluctuation in its yield this restorer must be subjected to further studies. The yield of Primepi, a restorer of French origin, was significantly lower in our experiments than the yield of the most productive variety.

The plant heights of the F_1 hybrids are given as a percentage of the height of Bezostaya 1 (Table 4). The data in the table reveal that the F_1 hybrids which exceeded the highest yielding varieties in productivity were 10—20% taller than the Bezostaya 1 plants. Further, it can be established from the three year average that the tallest F_1 hybrids were produced with the restorer Rf Mir.808, followed by Primepi, while the shortest ones originated from Rf (ms Mir.808 × Prof. Marchal). The same order of succession is obtained if the restorers themselves are classified according to height. The average plant height of Rf Mir.808 was 23.6% greater and that of Primepi 12.7% greater than that of Bezostaya 1, while Rf (ms Mir.808 × Prof. Marchal) was the same height as Bezostaya 1. The average plant height of Bezostaya 1 was 101.3 cm in 1973, and 118.1, 110.5 and 103.6 cm, respectively, in the successive years.

F_1 hybrids were also produced on the male sterile analogue of the dwarf wheat variety Krasnodar 1 with each of the restorers mentioned. An analysis of the heights of these hybrids shows that our statement concerning the plant heights of both the other F_1 hybrids and the

Table 4

Plant heights of F_1 hybrids compared to that of Bezostaya 1
(%)

Year	♂	Rf Mir. 808		Rf (ms Mir. 808× Prof. Marchal)		Primepi	
1972—1973	♀	ms Bez. 1	119.8			ms Skor. 35	115.6
1973—1974		ms Bez. 1	120.3	ms Avrora	110.5	ms Jub. 50	121.7
1974—1975		ms Bez. 1	117.9	ms Kavkaz	112.4	ms Jub. 50	119.0
1975—1976		ms Kavkaz	120.8	ms Bez. 1	120.2	—	—
Mean			119.7		114.4		118.8
1973—1974	♀	ms Kras. 1	108.4	ms Kras. 1	102.1	ms Kras. 1	103.2
1974—1975		ms Kras. 1	101.2	ms Kras. 1	94.4	ms Kras. 1	97.5
1975—1976		ms Kras. 1	107.9	ms Kras. 1	100.9	—	—
Mean			105.8		99.1		100.3

restorers holds true here too, though it should be added that due to the decrease in the height of the mother plant the height of the F_1 plants also decreased by 12—16%. The F_1 plant obtained by crossing ms Krasnodar 1 with Rf exceeded Bezostaya 1 in height only slightlys if at all.

According to our measurements the height of the wheat variety Krasnodar 1 and its male sterile analogue was 63.5—68.2 cm.

To sum up, by crossing certain cytoplasmically male sterile plants with restorers in small plot experiments F_1 hybrids were obtained whose yield exceeded by 20—30% the yield of the best commercially produced standard varieties included in the experiment. However, the plant height of the best F_1 combinations exceeded that of Bezostaya 1 by 10—20%. By using the male sterile analogue of Krasnodar 1 to produce F_1 hybrids, plants of the desired height can be obtained. The productivity of the Krasnodar 1 parent partner is not satisfactory, however, so its F_1 hybrids also yield less than the other F_1 hybrids in general.

As for the French restorer Primepi, its F_1 hybrids gave the lowest yields in our experiment. Primepi was found to have excellent restoring ability (this is in agreement with the literary data), but because of its other unfavourable agronomical properties it has no future as an F_1-producing partner.

A yield comparison of the restorers and the F_1 hybrids reveals that apart from restoring ability yield reliability is a highly important factor which must be considered when producing restorers.

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Prepared at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár

E. Rajki, J. Császár

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ROLE OF TITANIUM IN THE LIFE OF PLANTS I.

Titanium is one of the most frequent elements in the Earth's crust. Consequently, most soil samples brought to white heat contain 3—4 thousand ppm titanium, and in some soils of volcanic origin (in Hawaii) the titanium content amounts to 20 thousand ppm. In the soil the titanium is generally bound to silicates, so it is practically unavailable to plants. Some authors have demonstrated 1—30 ppm titanium in plant samples, but according to the majority of opinions expressed in the literature (CHAPMAN 1966) the titanium content must be considered in such cases as a consequence of soil pollution.

The international literature of the last decades includes about 500 scientific publications related in some form or other with the biological role of titanium, but hardly any of the papers refer in sufficient detail to the actual role of titanium, or give any sort of physiological evaluation. During the last twenty years an increasing number of technical books and monographs have been published on the biochemical and physiological roles of the elements (SCHARRER 1955, BOWEN 1966, EPSTEIN 1972, EICHORN 1973), but none of them describes titanium as a biologically important element.

In one of the first publications to mention the importance of titanium in plant physiology (TRAETTA—MOSCA 1913) titanium is said to promote the synthesis of chlorophyll. A very interesting paper was published by KONISHI—TSUGE (1936) giving an account of the favour-

able effect of potassium titanate on the development of lucerne, and on the number and activity of the root nodules. On this basis ANDERSON (1951) suggested the possible role of titanium in the nitrogen fixation process of leguminous plants.

According to POLUZEROV (1965) titanium shows intensive biological accumulation and poor soil migration properties. Doubt is cast on this statement not only by earlier literary data but also by the investigations of GLAZOVSKAYA (1964), who found the biological accumulation coefficient of titanium to be one of the lowest. Though there are many contradictory data, the statement (TONKONOZHENKO—KHLYUPINA 1974) that plants extract 75—2700 g/ha titanium from the soil seems to be the most extraordinary of all.

Data on the phytotoxicity of titanium are also interesting. It is difficult to understand the data of KUSAKA *et al.* (1971), according to which 30 ppm titanium in the soil disturbs the growth of turnip, while 150 ppm is totally phytotoxic. On the other hand, LUZZATI (1963) reported that in neutral and acidic soils titanium is only toxic at above 400 ppm. In our opinion the total amount of titanium in most soils is much higher than the values mentioned, while the soluble or available quantity of titanium is considerably lower.

A solution culture containing readily soluble salts naturally represents different conditions. HAAR *et al.* (1976) carried out solution culture experiments with cabbages and found that phytotoxicity began above a concentration of 1 ppm. This is in good agreement with the results of an earlier experiment on tomato test plants (PAIS *et al.* 1969b).

With respect to grain crops mention should first be made of the data of ASMAEVA—ILVITSKY (1969) who found 0—0.8 ppm titanium in the ashes of wheat grains. Considering that the ash percentage of the wheat grains is rather low, this amount, when related to dry matter, means a very low titanium concentration (about 0.01 ppm). MROWCA (1973, 1974) has recently patented various derivates of titanium and zirconium cyclopentadienyl. According to the description, under the influence of such titanium compounds the yields of wheat and pea increased by 15—20%. As regards these compounds it should be noted that they are complicated to produce, so there is not much hope of being able to utilize them economically.

A relatively early publication in connection with horticultural crops is the paper by TROITSKY (1955), who studied the composition of the soil, of the vines grown on them, and of the wines made from the grapes in various wine regions of the Soviet Union. According to his data an average of 2000 ppm titanium can be found in the soils, a maximum of 10 ppm in the grapes, and a few tenths of ppm in the wines. Organoleptic tests revealed a favourable effect of titanium in the wines on the development of flavour and aroma. DOBROLYUBSKY (1961) used titanium(III)-solution to spray vines, and found it to increase the volume of yield, raise the sugar content of the grapes and reduce the titrable acid content of the grape-juice. Our own experiments, which were of a somewhat different nature and will subsequently be described in detail, led to similar results.

Interesting and important experiments were carried out by RUTSKAYA (1971, 1976) and RUTSKAYA—RÜZHABSKAYA (1974) who found that the development of sugar-beet was favourably influenced by the addition of ammonium titanyl sulphate to the soil. According to their results the chlorophyll content of the leaves increased and the sugar content of the beet became higher. Our own experiments, carried out for several years with water-soluble titanium(IV)-compounds, have yielded results which are similar in many respects, but there are two important differences between the experiments: first, foliar nutrition was applied and secondly a concentration of only 3—15 g Ti/ha was applied instead of 300—900 g Ti/ha.

The earlier literary data are confirmed by the most recent Japanese patent specifications (WAKAMOTO 1973, TSUKAMOTO 1975), according to which fertilizer additives consisting of iron and titanium compounds check the process of denitrification, thus promoting the nitrogen turnover of the soils, and they exercise a favourable effect on the quantity and quality of soybean yield.

Very interesting are the investigations recently made by GRIZHANKOVA—BOYCHENKO (1975) in which titanium compounds whose chemical composition suggested they might be suitable for playing a biological role were isolated from some marine plants (e.g. *Laminaria japonica*, *Zostera marina*). These authors succeeded in producing titanium compounds analogous with pantotenic acid. No mention is made, however, of a possible biochemical effect, apart from the intensive reducing ability of these compounds.

In the autumn of 1968 work was begun on the elaboration of a research method suitable for objective investigations on the role of the microelements, including titanium (PAIS *et al.* 1969a). The essence of the method was that the container used to grow the plants in, and the medium used to fix the plants were synthetic materials (polyethylene and polystyrol, respectively) which are indifferent from the point of view of ion exchange. In addition the solution culture were prepared from ion-free water and chemicals of specific purity. The material of the containers and the purity of the solutions were controlled by a highly sensitive mass spectrographic technique.

In the very first experiments, besides the macroelements primarily required for the life of the plant, the effects of not only those micronutrients which are unequivocally considered essential in the literature, but also of some other elements, including titanium, were examined. The result of our experiments was that in tomato, which was used as the test plant a more favourable chlorophyll formation was observed as a response to titanium, and a higher margin of phytotoxicity was found compared to other microelements. In the case of cobalt and nickel 1 ppm proved toxic, while with titanium the same effect appeared at 5 ppm.

Between 1972 and 1974 foliar nutrition experiments were carried out with tomato in the field (PAIS—HODOSSI 1975). An increase in yield, favourable changes in composition and an acceleration of ripening were found. The leaf spray made up for these experiments contained a number of macro- and micronutrients, including titanium.

In spring 1974 regular, multilateral experiments were started to clarify the role of titanium in plant physiology and to study the possibilities for utilizing it in crop production. On the Kecskemét-Szikra State Farm three important horticultural plants, tomato, winter apple and vine, were sprayed with a 1 ppm titanium solution. In preparing the solution titanium tetrachloride of analytical purity was used and the dilution was made with ion-free water; in our experience titanium does not precipitate in solutions of such a low concentration.

In average samples from plots sprayed on three occasions the parameters of several components and the chlorophyll content of the leaves were measured. As seen in Table 1, foliar nutrition with titanium solution yielded highly favourable results in all three plants.

Table 1

Experiments with titanium solution used in foliar nutrition, 1974

Sample		Chlorophyll, mg/g	Refraction, %	Glucose, %
Újbög, tomato	treated	1.97	5.00	3.23
	control	1.53	4.50	2.24
Nyárlőrinc, Jonathan apple	treated	2.60	13.80	8.39
	control	2.06	13.00	7.80
Nyárlőrinc, Ezerjó grapevine	treated	1.33	15.70	13.40
	control	1.14	14.80	12.84

The very important role played by titanium in the life of plants is also suggested by the results obtained by mass spectrography on the leaves of plants sprayed with titanium solution (PAIS 1974). The data in Table 2 indicate that the favourable effect of titanium is

Table 2

Mass spectrography of ash prepared from leaves of test plants

Sample		Ti	Zn	Mn	Cu	Ni	Co	Cr
		ppm						
Jonathan apple leaf	treated	210	350	8400	410	26	6.5	7.5
	control	43	83	1400	120	5	1.9	1.7
Ezerjó vine leaf	treated	72	2810	3440	4120	24	2.3	2.8
	control	19	1160	1080	1790	10	2.1	1.0

also manifest in the marked increase in the concentration of some essential microelements. As an explanation it is assumed that titanium enhances the photosynthesis, and thus the metal ion demand of the increased enzyme activity which ensures this stimulates an increased ion uptake by the plant.

On the basis of our experimental results it was hoped to use foliar nutrition with titanium solution to solve an important problem facing sugar production in Hungary. In the summer of 1975 sugar-beet in three state farms was sprayed on three occasions with a solution containing titanium at a concentration of 1 ppm so that the plants received a total of 3 g/ha titanium. Particularly noteworthy results were obtained in the Mezőhegyes State Farm (PAIS *et al.* 1977). According to the evidence of the data shown in Table 3 the chlorophyll content of the leaves, the volume of root yield and the digestion value characteristic of the sugar content increased significantly.

Table 3

Effect of foliar nutrition with titanium solution in sugar-beet

Treatment		Chlorophyll, mg/g		Root weight, g/n		Digestion, %	
Mezőhegyes, 1975	treated	0.72	$SD_{5\%} = 0.10$	936	$SD_{5\%} = 158$	12.2	$SD_{5\%} = 0.5$
	control	0.60		754		10.4	
Mezőhegyes, 1976	treated	1.07	$SD_{5\%} = 0.23$	801	$SD_{0.1\%} = 25.5$	11.2	$SD_{0.1\%} = 0.82$
	control	0.79		662		10.0	

Only a brief mention will be made here of the highly favourable results obtained in three years of experiments with vine and lucerne as the test plants. A detailed account will only be given of the results attained when applying foliar nutrition to sweet corn.

On Kalocsa State Farm large-scale experiments were carried out in 1976 with two American varieties (Yukon and Commander) and in 1977 with a later ripening American variety (Merit), each with three replications. As seen in Table 4, the titanium solutions with various concentrations used in 1976 showed no significant differences in their effects. Therefore,

Table 4

Effect of foliar nutrition with titanium solution in sweet corn, 1976

Treatment		Fresh ear weight, g	Deviation, %	Red. sugar, g	Deviation, %
Yukon	control	196		1.84	
	1 g Ti/ha	238	+21.4	1.89	+ 2.7
	5 g Ti/ha	258	+31.6	2.39	+29.9
Commander	control	187		0.74	
	1 g Ti/ha	212	+13.4	0.94	+27.0
	5 g Ti/ha	221	+18.2	1.08	+45.9

Table 5

Effect of foliar nutrition with titanium solution in sweet corn, 1977

Treatment		Fresh ear weight, g	Deviation, %	Red. sugar, g	Deviation, %
Merit	treated	297	+29.1	3.47	+50.2
	control	230		2.31	

in 1977 titanium was only used at a concentration of 1 ppm, which has given good results with other test plants; the data from these experiments are given in Table 5.

The experimental data unequivocally show that the volume of fresh crop as well as its sugar content increased by an average of 25—30% in each variety. This double effect holds the prospect of great economic advantage.

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Prepared at the Chemical Department of the University of Horticulture, Budapest

I. Pais, M. Fehér, E. Farkas

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GERMINATION STIMULATION BY MAGNESIUM, AND THE MECHANISM OF ACTION

The literature on seed germination offers different biochemical explanations for the germinating ability and for the decrease and stimulation of germinating vigour. CHING—DANIELSON (1972) found a correlation between the ATP content and the germinating ability of the lettuce seed. RIES—EVERSON (1973) and EVANS—BHATT (1977) established that the germinating vigour of wheat was a function of the weight and protein content of the grain. According to SUKALSKI (1968) maize with a low magnesium content has reduced (hardly 20%) germinating ability. Germination and germ growth in rice was found by WILLIAMS—PETERSON (1973) to depend on the alpha-amylase activity. Similar alpha-amylase dependence was demonstrated by VERBEEK *et al.* (1972) in barley. A correlation was detected by VOROBEV (1976) between the viability and phytase activity of rice. CHEN—PARK (1973) observed germination stimulation by gibberellic acid in barley. A similar result was obtained by AVETISYAN (1975) when germinating crepis seeds. It is clear even from the above, incomplete list that most of the authors only studied the effect of a single factor on germination, and very few tried to find correlation with other factors.

Starting from the results of SUKALSKI (1968), who observed reduced germinating ability due to magnesium deficiency, an attempt was made to stimulate germination by introducing exogenous magnesium, and also to throw light upon the action mechanism.

Germination was generally carried out in Petri dishes on wet filter paper, but in the case of maize and sugar-beet moist sand was used. For the germination seeds of nearly identical size (weight) were selected. For germination in sand, the seeds were planted in quartz sand previously washed with hydrochloric acid and distilled water, and moistened to 70% water capacity.

The seeds were placed in holes made with a dibble but were not covered with sand, so the germinated seeds were easy to count (SHMILLIÁR 1965). The plastic boxes used for germination were covered with perforated transparent PVC sheets. The slight loss of water was replaced daily. Overwatering was prevented by a perforation (as an outlet) on the side of the box at the height of the holes made with the dibble. The germination temperature was 20—25 °C; the number of replications was 12.

The seeds of each treatment were soaked in a 1% solution of magnesium sulphate, or magnesium citrate of the same magnesium concentration, for 2—24 hours in order to introduce the magnesium. In the ATP treatment soaking was carried out in a 0.1% solution and in the case of GA in a 10 ppm solution. The control seeds were soaked in distilled water for the same time as the treated seeds. In our experiments 5 hours was found to be the optimum soaking time.

In evaluating the germination results the germinated seeds were taken as a percentage of the notal number of seed sown, and the length (in mm) of the hypocotyl was recorded for the treated seeds and the control at the same time. In the case of maize and sugar-beet the germinating vigour was also determined and expressed as the ratio of seeds which germinated by the 3rd and 10th day, respectively. For sugar-beet the multigerm seed (glomerule) was regarded as one seed.

With bean and flax plants emergence experiments were also carried out at the experimental station of the Borsod Chemical Works, in small plots laid out on brown forest soil. Here the effect of magnesium was characterized by the percentage emergence as a function of the emergence time.

The amylase activity was first studied using FREELAND's (1972) quick, semi-quantitative method. The method is based on the readiness of amylase to diffuse from the seed into the agar gel. When the halved seeds are placed with the cut surface on a starch-iodine-agar

plate the disappearance of the blue colour indicates the amylase activity. The activity is taken as proportionate to the discoloured area.

The quantity of amylase was determined after WILLIAMS—PETERSON (1973) from the dry matter loss occurring in the course of germination. According to these authors the amylase activity in the first eight days of germination is positively (0.9) correlated with the dry matter loss. The amylase activity was therefore expressed as the proportionate loss in weight of the germinating seed.

Gibberellins (GA) were demonstrated after LEXANDER (1974) using both the lettuce biotest and thin layer chromatography. 50 g samples of germinating seeds were homogenised in the cold in a 2.25 g/100 ml solution of basic lead acetate and than extracted four times with ethyl acetate. Separation was carried out in a centrifuge at 4500 g. The ethyl acetate was evaporated to dryness at 30 °C in a vacuum and the residue was dissolved in 2 ml ethyl acetate. This solution was used for the GA biotest on lettuce seedlings and for the thin layer chromatography. With the lettuce biotest the stimulative effects of extracts prepared from the control and from magnesium-treated germs on the elongation of lettuce hypocotyls were measured. For thin layer chromatography Fixion 50×8 plates and a 70 : 25 : 5 mixture of benzol-butanol-acetic acid as running solution were used. After draing the plates were examined under UV light, and from the intensity of fluorescence and the size of the spots conclusions were drawn on the amoint of GA. A parallel run was made with GA_3 as an identification control.

The effect of magnesium sulphate treatments on the germination and germinating vigour of seeds is shown in Table 1. As can be seen in the table, the extent of stimulation caused by the magnesium treatment was 29% in sugar-beet and 13% in maize. The increase in the germinating vigour, though considerable in maize (17%), was particularly remarkable in sugar-beet (34%). It occurred to us that the stimulation may have been partly or totally caused by something other than the magnesium, as LEXANDER (1974) found that an increase in the —SH level induced faster growth in the shoots. The increased —SH level might be due to the sulphate ions.

Our investigations were therefore extended to include magnesium citrate seed treatments. The quickly germinating lettuce was used as test plant, so as to reduce the likelihood of —SH ions being produced from SO_4^{--}. The results of the experiment are summarised in Table 2. The table unequivocally proves that the stimulation was due to the action of magnesium. It should be noted that the citrate treatment resulted in stronger germs with thicker root-hair. After the germination experiments our interest turned to the effect of stimulation on emergence. The results of sowing experiments with bean, pea and lentil seeds are seen in Table 3. According to the data in the table the extent of stimulation was 28.6% in bean, 17.6% in lentil and 14.3% in pea.

Table 1

Germination of sugar-beet and maize seeds as a function of treatment and time, expressed as the percentage of seed germinated

Plant	∅ Control		Magnesium treated			Germinating vigor		
	3rd day	10th day	3rd day	10th day	Δ%	∅	Mg	Δ%
Sugar-beet	7	37	14	48	29	0.183	0.281	54
Maize	6	82	9	93	13	0.073	0.090	17
$SD_{5\%} = 3.9$								

In order to discover wnether seeds with higher endogenous magnesium contents germinated better an experiment was set up (HETZER—KISS 1977) with seed-producing sugar-beet mother plants, where the control plot was treated with N alone, while the trial plots were fertilized with N + Mg (Agronit). The results of the experiment are contained in Table 4. The table reveals that the seeds of plants where the soil was treated with magnesium are richer in magnesium and their germinative ability is also better. The data of Tables 1, 2, 3 and 4 give convincing evidence of the stimulating effect of magnesium seed treatment on germination. An explanation of the stimulation was then sought.

Table 2

Effect of different compounds of magnesium on the germination of lettuce, as a percentage of germinated seed

Day of germination	∅ Control	$MgSO_4$		Mg-citrate	
	%	%	Δ%	%	Δ%
1st	62.9	73.3	16.4	73.6	17.0
2nd	83.5	88.7	6.1	89.7	7.3
$SD_{5\%} = 5.1$					

Table 3

Effect of seed treatment with magnesium on emergence as a percentage of the total seed sown and as a function of time

Plant	Treatment	Measuring dates			Δ%
		1	2	3	
Bean	∅	1	55	70	—
	Mg	25	80	90	28.6
Lentil	∅	13	55	67	—
	Mg	31	72	79	17.6
Pea	∅	60	62	70	—
	Mg	65	68	80	14.3
$SD_{5\%}$		—	—	4	—

Table 4

Effect of endogenous magnesium content increased by magnesium fertilization on the germination of sugar-beet seeds

Fertilizer	Mg content		Germinating ability	
	mg%	Δ%	%	Δ%
N	444	—	63.0	—
N + Mg	460	3.6	67.5	7.1
$SD_{5\%}$	9.8	—	4.0	—

The germination of the seeds and the growth of germs are functions of the quantity and activity of their alpha-amylase content (VERBEEK *et al.* 1972). On this basis it was assumed that under the influence of the magnesium treatment the activity of alpha-amylase would increase. The amylase activity was first examined with FREELAND's (1972) semi-quantitative test in maize and pea seeds. The magnesium treatment was found to increase the amylase activity. Quantitative analysis was then carried out after WILLIAMS—PETERSON (1973) by measuring the dry matter loss occurring in the course of germination. This time-consuming series of analyses was onlyar ried out on maize. The average data are summarized in Table 5, and clearly show that th mylase activity is substantially higher in seeds treated with magnesium.

Table 5

Alpha-amylase activity in maize (MvSC 580) as a function of treatment and time, expressed as percentage dry matter reduction

Age of germ (days)	Treatment		Δ%
	∅	Mg	
3	3.7	4.2	13
4	4.7	6.4	36
5	7.4	10.3	40
$SD_{5\%} = 0.8$			

The next task was to find out how the amylase activity was stimulated by the magnesium. The direct stimulation of the activity could not be demonstrated by means of the starch-hydrolysing ability of a pure amylase preparation. It was thus assumed that the magnesium increased the hydrolysis by raising the enzyme level through a de novo synthesis rather than by stimulating the activity of the amylase enzyme molecules. So our further investigations were directed towards the stimulation of amylase synthesis.

JACOBSEN—VARNER (1967) found that gibberellic acid (GA) stimulated the synthesis of amylase. This result is in agreement with the observations of CHEN—PARK (1973) and AVETISYAN (1975) with respect to the stimulation of germination by GA. A relation between magnesium and GA was then sought. The GA biosynthesis described by LANG (1970) requires magnesium and ATP in the first nine steps. The kaurenne cyclization from geranil-geraniol-pyrophosphate is particularly energy-intensive. This led to the conclusion that the GA synthesis can be stimulated either with ATP or with magnesium. To support this theory lettuce seeds were treated with magnesium (1% solution of $MgSO_4$), ATP (0.1% solution), with a mix-

Table 6

Effect of different seed treatments on the growth of lettuce hypocotyl

Treatment	∅	Mg	ATP	ATP + Mg	GA
Growth, mm	5.6	9.0	6.7	9.8	9.3
$SD_{5\%} = 0.7$					

ture of the two and with GA (10 ppm solution). The effect was measured by the elongation of the lettuce hypocotyl. The results are contained in Table 6. The data in the table prove that germination and the growth of the hypocotyl were stimulated by each of the treatments. It is worth noting that in the case of the ATP + Mg treatment the effects were additive.

These results only confirm the efficiency of the treatments but do not prove the stimulative effect of magnesium on GA synthesis. The latter was proved by determining the GA content in the control and in the magnesium-treated germinating seeds. Biotest and chromatography were both used for the determination. The GA was extracted from maize seedlings and the measurements were carried out as described by LEXANDER (1974). The extract from the control germs induced a 9.6 mm growth, and that prepared from magnesium-treated germs a 12.8 mm growth in lettuce hypocotyl, that is, an approx. 33% stimulation was obtained. With thin layer chromatography the most intensive greenish-blue fluorescence was observed at the 0.1 Rf spot corresponding to GA_3 (control). The other spots (with different Rf values) were hardly perceptible (and thus could not be evaluated). The size of the fluorescing spots and the light intensity difference of the fluorescence convinced us that the magnesium treatment really stimulated the synthesis and level of GA. A similar rise in the GA level was indicated in another magnesium experiment carried out with white mustard, pea and bean as the indicator plants. Of the results of these experiments it is sufficient to mention the growth of the leaf area (in long-day mustard grown under short-day conditions), the earlier flowering (after 21 days), and the increased number of yield components.

It may be interesting to note that different wheat varieties obtained from the same growing site gave different germination results (Table 7). The differences are explained by the variety dependence of the GA stimulation, as pointed out by PALEG (1960) for barley (varieties Baku and Prior).

Table 7

Germination stimulation with magnesium in wheat an a function of variety, expressed as percentage germination

Treatment	Jubileinay	GKK3	Bezostaya 1
∅	81.4	88.2	88.1
Mg	87.8	92.1	89.7
Δ%	7.8	4.4	1.8
$SD_{5\%} = 3$			

The germination of seeds is thus related to their magnesium contents, and germination can be stimulated by the application of exogenous magnesium. A similar stimulation of germination was obtained by raising the endogenous magnesium content through soil fertilization. The stimulation was found to be equally effective for both starch- and oil-containing seeds, and for those rich in protein. The extent of stimulation depends on the species and the variety, and, in our opinion, on the original (endogenous) magnesium content as well.

Magnesium exerts its stimulative effect on germination in three ways: *a)* by increasing the energy charge of adenilate (ATP formation), *b)* by stimulating GA synthesis, and *c)* through protein synthesis (enzyme formation).

a) Increasing the adenilate energy charge. According to our measurements the germination of seeds can be stimulated by introducing exogenous ATP. This statement agrees with the data of BROWN (1965), who found that the low ATP level of the dry seed increased three-

fold during swelling and germination did not start until it reached a critical level, depending on the plant. On the basis of the formula (ATP + 1/2 ADP) : (ATP + ADP + AMP) this critical level is 0.78 for *Acer sacharinum*, and 0.58 for *Pisum sativum* (SIMMONDS—DUBROFF 1974), to give only a few examples. Whather ATP synthesis takes place through photophosphorylation or mitochondrially. it is inconceivable without magnesium (GROSSE 1963). According to our own measurements the pyrophosphate-chelate concentration can be increased by magnesium application (KISS—POZSÁR 1976). Thus, an improvement (to a certain degree) in the magnesium supply of the seed stimulates an increase in the adeniate energy charge (ATP level). When the adenilate charge has reached a critical level, both hormone (e.g. GA) and protein synthesis begin, thus inducing germination to start.

b) Magnesium increases the GA synthesis partly by helping to attain the necessary ATP level, and partly by facilitating energy transmission. The GA synthesis demands a great deal of energy (LANG 1970). This is one reason why it requires magnesium, which helps in the energy transmission and is also the enzyme activator of the GA synthesis. It is in these two ways that magnesium stimulates the synthesis of GA.

c) By stimulating protein synthesis the magnesium exerts an effect on the development of the seedling in two ways. Stimulation through the synthesis of alpha-amylase (and of the enzymes in general) is an indirect way. It has been proved experimentally that the stimulation of the amylase activity is due to an increase in concentration through de novo synthesis, and is not the result of molecular activation. The synthesis of amylase (as a protein) is equally stimulated by magnesium and GA. Magnesium promotes this synthesis by maintaining the polyribosome structure as well as through energy transmission (WETTSTEIN *et al.* 1963). GA increases the protein synthesis by stimulating the synthesis of RNAs (LESHEM *et al.* 1976). This stimulation of the RNA synthesis is again attained through the enhancement of the polyribosome formation (RAO—KHAN 1975). The second may is the stimulation of protein synthesis, the building blocks of the seedling, again through the maintenance of the polyribosome structure.

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Prepared at the Borsod Chemical Works, Agrochemical Section, Kazincbarcika

A. S. KISS

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CAUSES OF THE SPREAD OF WHEAT POWDERY MILDEW [ERYSIPHE GRAMINIS DC F. SP. TRITICI (MARCHAL)] IN HUNGARY AND THE POSSIBILITIES OF CONTROLLING IT

Powdery mildew is a disease which has been known for a long time in Europe; de Candolle described the pathogen in 1815. According to MEZEY (1894) it was already causing yield losses in Hungary in the last century. Owing to the local nature of the infection the yield losses were not considerable at that time. This favourable situation was maintained until the sixties of this century. Since then, however, a radical change has occurred in the distribution of the pathogen, as proved by the work of PODHRADSZKY—CSUTI (1962) which reported on the powdery mildew epidemic in 1961. From the beginning of the seventies the infections and epidemics have become more frequent. Nowadays infection by powdery mildew and consequent yield losses are regularly reckoned with. The increasing problems and the reduced yields urge us to deal with this question in detail. This is why this paper first discusses the causes of the pathogen's rapid spread and the possibilities of control, after which the results of fungicide experiments in 1976 and 1977 are presented.

Temperature and humidity. MANNERS—HOSSAIN (1963) established the optimum temperature (20 °C) and relative humidity (100%) required for the rapid multiplication of the pathogen. According to their observations, however, 50% of the conidia germinated even

at 50% relative humidity, though in that case the rate of both germination and growth slowed down. LAST (1963) studied the correlation between the length of the incubation period and the temperature. In his experiments incubation lasted for 3 days at 20 °C, and for 14 days at 5 °C. The above data prove that there are temperature and humidity optima for the reproduction of the pathogen. If these are given, an epidemic easily develops.

Infection. According to the investigations of NOVER (1966, 1968) the infection is mostly caused by conidia and less frequently by ascospores. With regard to the latter HERMANSEN (1964, 1968) writes that these are designed to prevent a break in the "chain of infection" during the summer. The conidia are sensitive to high temperatures; in hot weather they lose their viability within a few days. The ascospores are tolerant to high temperatures and therefore have an important role in infecting the second growths and new stands. Yet it is the conidia, which are carried by the wind to great distances, that primarily ensure the rapid spread of the pathogen. According to the investigations of BLUMER (1967) there is a large number of conidia. He found 5000—6000 conidia per 1 mm^2 diseased leaf surface.

Susceptibility, resistance. Various authors are mostly of the same opinion concerning differences in powdery mildew susceptibility between the varieties. In experiments carried out in Yugoslavia POTOCANAC—JAVOR (1973) found the varieties Leonardo, Libellula and Bezostaya 1 to be susceptible. According to SCHMIDT (1975) in Czechoslovakia Avrora, Bezostaya 1, Mironovskaya and Jubileinaya 50 are susceptible, while Mironovskaya 808 is less susceptible to powdery mildew. In experiments carried out by OBST (1974) in the German Federal Republic the varieties also differed in susceptibility to powdery mildew. Kranich, Benno and Caribo proved susceptible. Investigations in Hungary also confirmed the different susceptibility of the varieties. PODHRADSZKY—CSUTI (1962), for example, consider that the production of susceptible varieties was partly responsible for the epidemic in 1961. MANNINGER (1971) supposes that the rapid spread of the pathogen in Hungary was promoted by susceptible varieties being grown on large areas. According to SZUNICS *et al.* (1974) Bezostaya 1, Martonvásári 2, Martonvásári 3, Avrora, etc. are highly susceptible to powdery mildew. On the basis of his examinations BALLA (1975) has arrived at the conclusion that there are no resistant varieties among those commercially produced in Hungary; they are all more or less liable to infection. This statement is confirmed by SZUNICS *et al.* (1976) who report that most varieties grown in Hungary are susceptible, so that this factor in the development of an epidemic is given. The authors note, however, that in their experiments the varieties Arthur and TP 114/65 A proved resistant and can therefore be used as a source of resistance. WOLFE—BARRETT (1977), on the other hand, point out that because of the appearance of new races the resistant varieties lose their resistance or tolerance after a certain length of time.

Powdery mildew races. MARCHAL (1902, 1903) demonstrated special powdery mildew forms of the host plants of the pathogen at the beginning of this century. Many works have been published on the specialization of powdery mildew GORLENKO 1951, NOVER 1958, etc.). The biological forms of the pathogen have been discussed by HUSZ (1951) and GOLOVIN (1960). According to WATERHOUSE (1930) and MAINS (1933) the biological forms are not uniform; they can be divided into races. NOVER (1966) isolated 50 races from wheat and barley up to the middle of the sixties. According to MRÁZ (1972) many races occur in Czechoslovakia too, of which No. 0, No. 3 and No. 18 are the most frequent ones. SZUNICS—SZUNICS (1975) produced 638 pure lines of powdery mildew over a 5-year period and differentiated 35 races from them. They were the first to isolate and describe races No. 46, 47, 48, 52 and 53. On the basis of the above it can be established that the new races also promoted the rapid spread of the pathogen.

Crop rotation and monoculture. ANONYMOUS (1969) points out that wheat monocultures, combine harvesting and infectious leaf remnants are also causes of the wide distribution of powdery mildew. According to BOCKMANN—KNOTH (1971) and ZWATZ (1975) one-sided cereal

production creates favourable conditions for foot-diseases, while a wheat monoculture also promotes powdery mildew.

Artificial fertilization and rates of application. Some authors (PAQUET 1968, BROUWER 1972, POKACKA—BLONSKA-PAWLIK 1973, SZEPESSY 1977) explain the rapid spread of the pathogen by an excessive and disproportionate use of nitrogen fertilization. GLYNNE (1958) emphasizes the favourable effect of potassium, since in his experiments the extent of infection ranged between 26.7 and 44% without potassium, and fell to 0.2% when potassium was supplied. SCHÜTTE (1967) stresses the importance of boron, among the microelements, because when it is absent the degree of infection increases. According to infestigations in Hungary high rates of nitrogen combined with relatively small amounts of phosphorus and potassium are particularly disadvantageous because they increase the danger of lodging and create favourable conditions for various pathogens.

Plant stand, plant density. According to JANKE (1970), BROUWER (1972), UBRIZSY (1965) the degree of infection in overdense stands is higher than in those with an optimum nnmber of spikes. With a plant number resulting in 500 spikes per m^2, the varieties grown at present in Hungary give maximum yields, and 600 spikes per m^2 are sufficient even for varieties of southern origin. In the current Hungarian varieties it is therefore disadvantageous to increase the number of spikes.

Sowing time. From his investigations JENKYN (1976) arrived at the conclusion that stands sown too early were more infected than those sown on the optimum date. According to experiences gained in Hungary stands sown in September soon become infected when the autumn is prolonged. If a long autumn is followed by a mild winter and an early spring, the conditions for an epidemic already exist at the end of March.

Yield loss. In experiments carried out by LARGE (1963) in England powdery mildew caused a 1.25 q/ha yield loss over a 3-year average. According to BROUWER (1972) yield losses may amount to 20% or more. The author explains this as a reduction in the assimilating surface, a decrease in standability and the formation of empty grains. NOVER (1966) estimates the grain loss at 10—15%. MRÁZ (1971) thinks the extent of damage is dependent on the susceptibility of the varieties, as in his experiments the yield of the variety Consul decreased by 18.1% and that of Remoé by 10.3%. BRÖNNIMANN (1974) found the extent of the losses to vary with the degree of infection. If the leaves are only slightly infected or if the infection occurs at a later stage of development and the uppermost leaf (flag) remains intact, there will be no yield loss. In the opposite case the yield losses are considerable. LARGE—DOLING (1962) also draw attention to the role of the flag leaf, as in their experiments it supplied the skike with some 50% of its assimilates, while the other three together supplied the remaining 50%. In their opinion the damage is not considerable when this leaf gets infected very late or is not affected BAJLA (1975) does not consider powdery mildew to be a dangerous disease either. He writes, among others things: "The damage done by powdery mildew is far from being as great as might be expected from the extent of infection. And catastrophes caused by powdery mildew are not known in the literature at all, not even in countries where the conditions are more favourable for the disease than in Hungary." SZUNICS—SZUNICS (1970a) found no yield loss up to 30% infection. In a later paper SZUNICS *et al.* (1976) showed that the extent of the yield loss depended on the susceptibility of the varieties, the date of appearance of the pathogen and rate at which the diesease spreads.

On the basis of the above it can be established that yield losses vary according to the climatic conditions, variety, etc.

Control. The possibilities of control naturally follow from the above. They are: the cultivation of less susceptible varieties, crop rotation or at least a healthy succession of crops; harmonious NPK fertilization; not too early and not too dense sowing. In years favourable for the development of a powdery mildew epidemic these methods of control are not, however,

sufficient; fungicides must also be used. The necessity for chemical control can be decided on the basis of the extent of infection. According to PARMENTIER (1973) fungicides are needed when 25 powdery mildew colonies (pustules) are found on the 3 upper leaves of 40 wheat plants. In practice this means that spraying must start at the very beginning of the infection. GEISSLER *et al.* (1975) drew the same conclusion from investigations in this field. Many fungicides have so far been tested in chemical control operations. LARGE—DOLING (1962), NIEMANN (1964), ZWATZ (1966, 1973, 1974, 1975), NOVER (1966), KRADEL *et al.* (1969), PÜZHIKOVA (1970), BAUERS (1971), KASPERS (1969), KASPERS—KOLBE (1971), POMMER—KRADEL (1971), PÁSTI (1972), DIERCKS (1973), FOSCHI—SVAMPA (1973), GYÖRGY—ERDEI (1974), OBST (1975) and KOLTAY (1975) found a considerable proportion of the examined fungicides to be suitable for the control of the pathogen.

In experiments at Martonvásár during the sixties the effects of Morestan, PP 149, PP 781, and later the action of Calixin were studied. The results obtained were only partly satisfactory, as the fungicides remained active for about two weeks, after which the wheat was reinfected. In the case of a single treatment there were no significant differences between the yields. Repeated control operations, on the other hand, are worth considering from an economic point of view. Calixin has a somewhat longer term of action (20 days or so), but because of its phytotoxic effect, as a result of which it decreased the yield in two of the three years of examinations, research was discontinued (KÜKEDI 1977). The new, more effective fungicides were found, however, to be worth testing further. An account of the results of these experiments is given below.

The experiments were carried out at Martonvásár, at the Agricultural Research Institute of the Hungarian Academy of Sciences, on a chernozem soil with forest residues, in 1976 ant 1977. The treatments were arranged in a random block design with 4 replications, in plote of 18 m^2. Each treatment received 120 kg/ha NPK active ingredients a year. The test plant was the highly mildew susceptible Avrora in 1976 and the moderately susceptible Martons vásári 4 in 1977. The variety was changed because of the susceptibility of Avrora to foot-diseases.

In Table 1 the meteorological conditions are presented. This is necessary because of the decisive influence of the major climatic factors (temperature, precipitation) on the extent of infection and the development of epidemics.

Considering the temperature data for 1976, it can be established that in March the weather was not favourable for the rapid spread of the pathogen, since the monthly temperature mean was lower by 2.6 °C than the many years average (5.2 °C), so the infection practically

Table 1

Monthly average temperatures (°C)

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1976	0.5	—0.3	2.6	12.1	16.7	20.7	23.2	18.6	15.1	11.2	6.7	0.3
1977	—0.2	4.4	8.8	9.7	17.2	20.7	20.4	19.8	14.6	11.5	5.3	—0.5
Monthly amount of precipitation (mm)												
1976	44	5	25	60	20	27	20	39	97	54	42	2.6
1977	42	58	68	35	42	57	48	37	28	13	57	16

ceased. Later, in April, favourable weather conditions came about and at the end of the month the danger of an epidemic was very real. The fact that it did not ensue was due to the dry weather and low relative humidity in May and June. So in the first year of the experiment only a mild infection occurred.

In the second year (1977) the weather conditions required for the rapid multiplication of the pathogen were already found in March. The epidemic was expected to break out at the beginning of April, but was prevented by night frosts lasting for some two weeks. In the second half of the month the weather took a favourable turn again and the powdery mildew began to spread at a fast rate. Nevertheless, an epidemic did not occur, because in the first half of May the daytime temperature was 25—30°C, which forced the pathogen to stagnate. For these reasons the infection was only of medium extent in 1977 too. In the first year of the experiment (1976) the treatments were:

1. Untreated control	
2. Chinoin Fundazol 50 WP	1 kg/ha (Benomyl)
3. Thiovit	5 kg/ha (sulphur)
4. Tilt	2.5 kg/ha (mixture of Capta fol)
5. MKF-674	2 kg/ha (para-chlorophenylazocyanoacetic acid)
6. SF 101	1.5 kg/ha (0,0-diethyl-N-phthalinidophosphonothionate)
7. H-15	3.3 l/ha (Carbendazim)
8. Persulon	1 l/ha (Fluotrimazole)
9. Afugan	2 l/ha (Pyrazophos)
10. Thiovit	twice 5 kg/ha (sulphur)

The first spraying was carried out on 5th May 1976 at stage H and the second, in treatment 10 only, on 31st May, at stage N. In the second year the treatments were:

1. Control	
2. Afugan	2 l/ha (Pyrazophos)
3. Bayleton	0.5 kg/ha (Triadimephon)
4. Chinoin Fundazon 50 WP	1 kg/ha (Benomyl)
5. Saprol	1.5 l/ha (Triforine)
6. Persulon	1 l/ha (Fluotrimazole).

Spraying was carried out on 27th April and 20th May 1977. According to the Keller—Baggiolini scale the wheat was at stage I on the first and at stage N on the second occasion of spraying. In 1977 3 factors were studied in the experiment:

1. Fungicides (5)
2. Number of sprayings (2)
3. Time of spraying (2, stages I and N).

The 1976 experiment was mainly aimed at choosing the best fungicides. That year only one evaluation was made (10th June). For this purpose 10 plants were removed from each plot and evaluated separately for each leaf level. Since the experiment was carried out with 4 replications a total of 40 plants per treatment were evaluated.

In the second year (1977) the same method was used, with the difference that 25 plants (a total of 100) were removed from each treatment and these were scored on 2 occasions (15th May and 7th June). The following scale was used for scoring

1. No infection	0%
2. Very slight infection (1 per leaf)	2.5%
3. Slight infection (2—4 per leaf)	5.0%
4. Fairly light infection (approx. 5—9 per leaf)	10.0%

5. Medium infection (10—20 per leaf)	15.0%
6. Strong infection (more than 20 per leaf)	20.0%
7. Very strong infection (half of the leaves turned yellow)	35.0%
8. Very strong infection (most of the leaves necrotized)	67.0%
9. Total infection (all leaves necrotized)	100.0%

The scoring results are given in Table 2 and the trend of yield averages are shown in Table 3. Figs 1, 2 and 3 give details of the experiments.

Summing up the data of Table 1 it can be seen that the effect of the fungicides tested in 1975 ceased or decreased to a great extent a month after the application, with the exception of Persulon and the double treatment with Thiovit.

Table 2

Average scoring results for powdery mildew infection

1976

Treatment	Dose 1 kg/ha	Date of spraying	Date of storing	Degree of infection
1. Control	—	—	10th June	5
2. Chinoin Fundazol 50 WP	1	5th May	,, ,,	5
3. Thiovit	5	,, ,,	,, ,,	4.8
4. Tilt	2.5	,, ,,	,, ,,	5
5. MKF-674	2	,, ,,	,, ,,	4.8
6. SF-101	1.5	,, ,,	,, ,,	4.8
7. H-15	3.3	,, ,,	,, ,,	4.9
8. Persulon	1	,, ,,	,, ,,	4.5
9. Afugan	2	,, ,,	,, ,,	5
10. Thiovit (2×)	5	5th, 31st May	,, ,,	4.6

1977

Treatment	Dose	Scoring averages on				
		15th May	7th June	7th June	15th May	7th June
		A		B	C	
1. Control	—	3.7	4.8	4.5	3.8	4.7
2. Afugan	2	3.0	4.8	4.7	3.2	4.0
3. Bayleton	0.5	1.2	3.5	4.6	1.2	3.1
4. Chinoin Fundazol	1	3.0	4.7	4.3	2.9	4.2
5. Saprol	1.5	3.2	4.5	4.5	3.1	4.4
6. Persulon	1	1.4	3.8	4.1	1.5	3.3

Note:
In treatment A spraying took place on 27th April (stage I).
In treatment B spraying took place on 20th May (stage N).
In treatment C spraying took place on 27th April and 20th May (stages I + N).

Fig. 1. Control

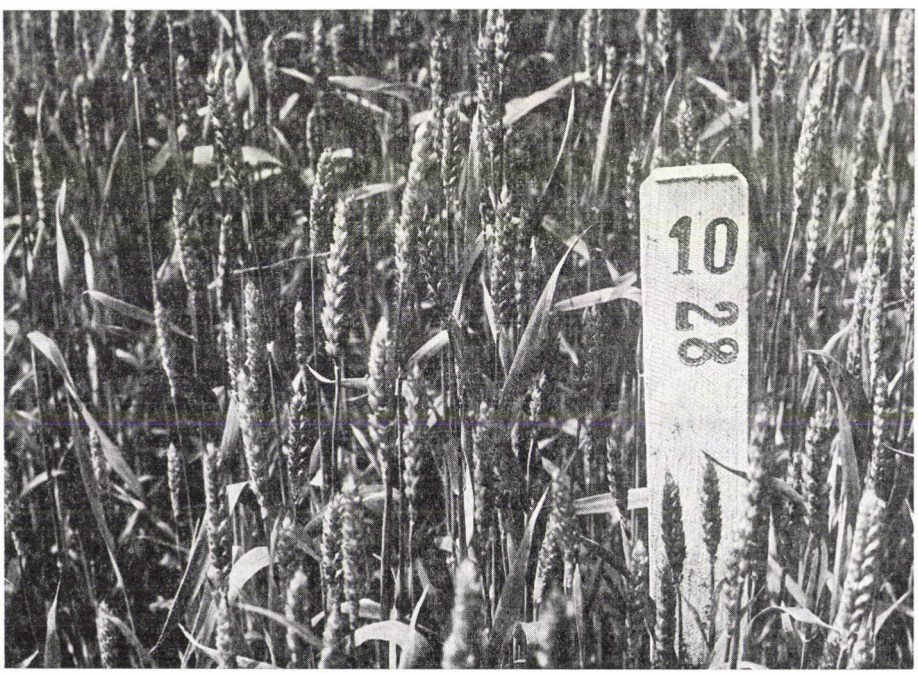

Fig. 2. Persulon 1 l/ha

Fig. 3. Bayleton 0.5 kg/ha

In the second year (1977) when spraying was carried out at stage I, the situation was similar to that in the previous year. After spraying with Afugan, Chinoin Fundazol 50 WP and Saprol the wheat was reinfected in about two weeks. The scoring results are very close to, or agree with those for the control. The effects of Bayleton and Persulon, on the other hand, lasted much longer than those of the above-mentioned fungicides.

The results obtained by spraying at stage N were also very poor compared to the control. The fungicides examined are thought to give considerably better results when used ar the beginning of the infection. Nor is it impossible that the effect of the fungicides was somewhat reduced by the rainfall that occurred a few hours after spraying. (The increase in yield was quite good in spite of the poor scoring results.)

In the C-treatment, where spraying was carried out on two occasions, at stages I and N, the results were also poor compared to the control.

After this analysis of the scoring results let us consider the data of Table 3. Looking at the yield averages in 1976 significant yield differences are found in the Thiovit, Tilt, MKF-674, Persulon and Afugan treatments compared to the control. Chinoin Fundazol 50 WP also increased the yield, but the difference compared to the control was not significant. No explanation was found for the adverse effect of Thiovit applied on two occasions.

In 1977, the second year of the experiment, the fungicides again increased the yields. The best effect was again shown by Persulon, followed this time by Bayleton. In these treatments the yield differences compared to the control were significant (treatments A and B). In treatment C, where spraying was carried out on two occasions, at stages I and N, no yield surplus was obtained compared to the results of single applications. In our opinion this can be attributed primarily to the medium or still lower extent of infection, when the uppermost leaf (the flag) remained almost completely intact and was thus able to fulfil its function of

Table 3

Effects of fungicides on yield averages in wheat (1976—1977)

1976

Treatment	Dose 1 kg/ha	Yield	
		q/ha	%
Control	—	54.61	100
Chinoin Fundazol	1	56.92	104.2
Thiovit	5	58.30	106.7
Tilt	2.5	58.20	106.5
MKF-674	2	58.56	107.2
SF-101	1.5	56.51	103.4
H-15	3.3	55.12	100.9
Persulon	1	61.53	112.6
Afugan	2	58.05	106.2
Thiovit (2×)	5—5	54.97	100.6
SD at 95%		2.80	5.1

1977

Treatment	Dose 1 kg/ha	Yield					
		A		B		C	
		q/ha	%	q/ha	%	q/ha	%
Control	—	51.50	100	51.11	100	50.38	100
Afugan	2	52.77	102.46	53.72	105.10	52.77	104.74
Bayleton	0.5	54.16	105.16	54.27	106.18	52.88	104.96
Fundazol	1	52.77	102.46	51.22	100.21	51.38	101.98
Saprol	1.5	53.16	103.22	52.77	103.24	49.83	98.90
Persulon	1	56.77	110.23	55.55	108.10	55.55	110.26

S.D. at 95% between fungicide treatments: 2.61 q/ha.
S.D. at 95% between spraying times: 2.38 q/ha.

Note:
In treatment A spraying took place on 27th April (stage I).
In treatment B spraying took place on 20th May (stage N).
In treatment C spraying took place on 27th April and 20th May (stages I + N).

supplying the spike with assimilates. The other cause was the weather, which was not at all favourable for the reproduction of the pathogen. With a scoring value of 6 or higher the situation would presumably have been different.

To sum up, it can be established that the spread of the pathogen in Hungary has mainly been caused by changes in the cultural practices. In addition, the problems caused by powdery mildew have also increased by the multiplication of new races. In the fight against powdery mildew help can be expected from the correct succession of crops, a harmonious NPK supply,

an optimum date of sowing, the proper number of plants per unit area, and last but not least the cultivation of less susceptible varieties.

However, in years with weather conditions favourable for the rapid spread of the pathogen fungicides must also be used, since these methods of control are not sufficient in this case. In our experience the optimum time for control is at the beginning of the infection (stages H-I). In years when epidemics occur a second application of fungicides (at stage N) is justified, as even the best fungicides do not remain active for more than 4—5 weeks. Of the fungicides examined Persulon and Bayleton showed the highest curative and eradicating effects, when used at rates of 1 l/ha and 0.5 kg/ha, respectively. Bayleton is effective against Puccinia spp. and Septoria too, and is thus considered superior to the other fungicides for the second application (at stage N).

*

Prepared at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár

E. KÜKEDI

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SOME ASPECTS OF USE OF ANEUPLOIDY IN WHEAT BREEDING

Aneuploidy in plants first became the subject of research when the first trisome was discovered in *Datura stramonium* (BLAKESLEE 1921). The first discovery was obviously only of theoretical importance; nevertheless it is to thate date tha the practical application of aneuploid genetics must be traced back. Today aneuploid plants have been found or produced in some 30 species of cultivated plants. In aneuploidy research cultivated common wheat *(Triticum aestivum)* plays the leading role. There are two reasons for this: 1. wheat has long been one of the most important sources of food in the world and is likely to be so for a long time to come; 2. in the wheat variety Chinese Spring SEARS (1954) produced valuable aneuploid lines (nullisomic, monosomic, trisomic, tetrasomic, telocentric, etc.) which are not available in other plant species. These lines serve as a basic material for the development of further

aneuploid lines in the varieties currently under cultivation, and they are also indispensable if a knowledge of the genetic structure of wheat chromosomes and the localization within the chromosomes of the characteristic features is to be acquired (MORRIS—SEARS 1967, LAW—WORLAND 1972, SUTKA 1973). It is by the use of this material that the cytogenetics of wheat has reached the present level where chromosomes and chromosome segments can be translocated as planned from one variety to another, and even from wild species to cultivated ones.

Aneuploidy in wheat

The hexaploid wheat (*Triticum aestivum*, 2n = 6x = 42) grown in Hungary is a complicated alloploid, with the genome constitution AABBDD. This means that the evolution of *Triticum aestivum* presumably occurred with the participation of three diploid species: *Triticum monococcum* — AA, *Aegilops speltoides* — BB and *Aegilops squarrosa* — DD. The theory that *Aegilops speltoides* was the source of the B genome is no longer accepted by most cytogeneticists; at the same time, no other species that could be regarded without any doubt as the source of genome B has been found so far (KIMBER 1974, JOHNSON 1975, HADLACZKY—BELEA 1975, GERLACH 1977, IORDANSKY *et al.* 1978, BELEA—FEJÉR 1979).

Genomes A, B and D in *Triticum aestivum* have a certain degree of relationship with each other. SEARS (1952) established this fact when crossing the tetrasomes with the nullisomes. When doubled (tetrasomes) certain chromosomes were found to be able to make up for the absence of others (nullisomes) so well that the hybrid plant could not be essentially distinguished from the disomic plants. On the basis of these experiments the 21 chromosomes of wheat have been placed in 7 groups (Table 1). Each group includes 3 chromosomes. Each

Table 1

Old and new symbols of wheat chromosomes (after OKAMOTO *1962 and* CHAPMAN—RILEY *1966)*

Homoeologous group	Genome A	Genome B	Genome D
1	(XIV) 1A	(I) 1B	(XVII) 1D
2	(II) 2A	(XIII) 2B	(XX) 2D
3	(XII) 3A	(III) 3B	(XVI) 3D
4	(IV) 4A	(VIII) 4B	(XV) 4D
5	(IX) 5A	(V) 5B	(XVIII) 5D
6	(VI) 6A	(X) 6B	(XIX) 6D
7	(XI) 7A	(VII) 7B	(XXI) 7D

of these 3 chromosomes is a member of one of the three genomes (A, B and D). The 7 groups of chromosomes, in which the 3 chromosomes are partly able to compensate for each other, are called homoeologous groups (OKAMOTO 1962).

Under normal conditions 21 bivalents are formed in the meiosis of the hexaploid *Triticum aestivum* (Fig. 1), that is, the individual chromosomes only pair with their homologous partners, while no pairing occurs between the homoeologous chromosomes belonging to different genomes. OKAMOTO (1957) and RILEY (1958), independently from one another, explained this phenomenon by a genetic control of pairing between homoeologous chromosomes. In

euhaploid wheat a maximum of 3—4 bivalents are formed, while the other chromosomes appear as univalents (Fig. 2); at the same time trivalents are also formed in the 5B nullisomic haploid (RILEY—CHAPMAN 1958). This observation has led to the conclusion that in the 5B chromosome a gene inhibiting homoeologous pairing is located. This theory was confirmed by an experiment in which the 5B monosomic line of *Triticum aestivum* was crossed with other wheat species (RILEY—KEMPANNA 1963). The cytogenetic analysis of the F_1 interspecific hybrids in the first metaphase of meiosis revealed that in hybrids where chromosome 5B was absent the frequency of occurrence of univalents was considerably lower and that of bivalents and quadrivalents higher than in those species hybrids in which the 5B chromosome was

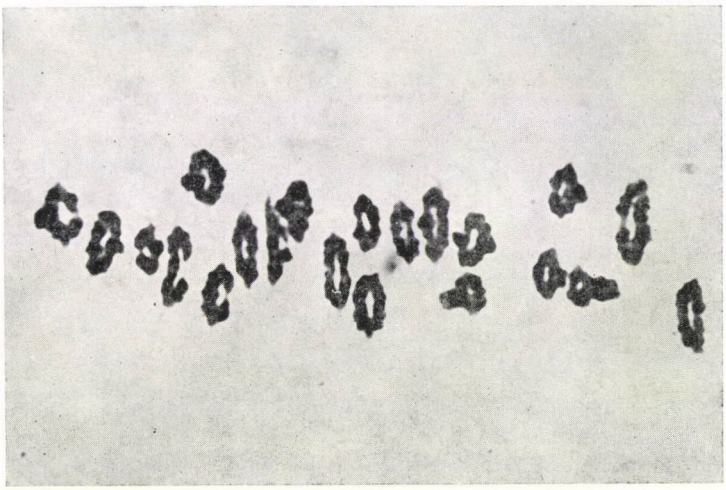

Fig. 1. First metaphase of meiosis in *Triticum aestivum* (21^{II})

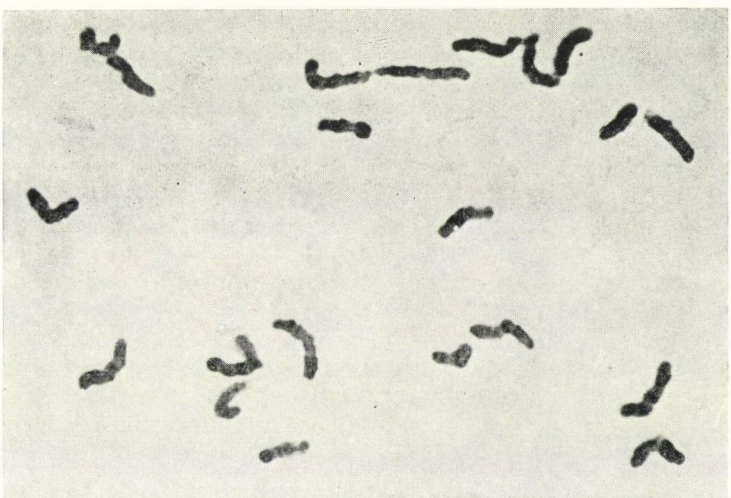

Fig. 2. Configuration of the metaphase chromosomes at meiosis in the haploid of Mironovskaya 808 (21^{I})

present. In 1965 Riley and Law localized the gene (Ph) responsible for preventing homoeologous pairing in the long arm of chromosome 5B (RILEY—LAW 1965). With mutagens a mutation suppressing the action of 5B can be induced (RILEY *et al.* 1966a).

FELDMAN (1966, 1968) studied the action of the gene found in chromosome 5B, which inhibits the pairing of homoeologous chromosomes but makes pairing possible for homologous chromosomes. When the dose of 5BL was increased six times the pairing of homologous chromosomes decreased, but heteromorphic bivalents were also formed, probably due to homoeologous pairing. According to Feldman's theory the extra dose of 5B decreased the premeiotic somatic association. Not only the homoeologous but also the homologous chromosomes are situated at random relative to each other. Some homoeologous may get closer to each other than the corresponding homologous.

In the genetic regulation of meiotic chromosome pairing other chromosomes also take part, though they have a weaker effect (SEARS 1976). Chromosome 5D, for example, stabilizes the meiotic pairing below a temperature of 15 °C (RILEY *et al.* 1966b). The suppressor found in chromosome 3D also has a considerable influence (MELLO-SAMPAYO 1971).

In further studies on the genetic control of meiotic chromosome pairing major progress is expected from the use of the Giemsa C-banding technique (METTIN *et al.* 1976, DHALIWAL *et al.* 1977).

Aneuploids may occur spontaneously in hexaploid wheat varieties with a frequency of about 1%, so such plants can be selected from the population by means of cytological examinations, but the screening is very time-consuming. Most wheat cytogeneticists use the aneuploid lines of Chinese Spring produced by SEARS (1954) to develop monosomic and substitution lines in other wheat varieties.

By using the Chinese Spring monosomic set it seems to be relatively simple to develop monosomic lines in other wheat varieties (UNRAU *et al.* 1956). All the 21 monosomic lines of Chinese Spring are crossed with the variety (variety B) in which the monosomic line is to be produced. Since the Chinese Spring monosome develops two types of gamete (n = 20 and n = 21), and the B variety only one type (n = 21), some of the progeny will be monosomic and the others disomic. In the next step the monosomic F_1 plants are crossed back to the

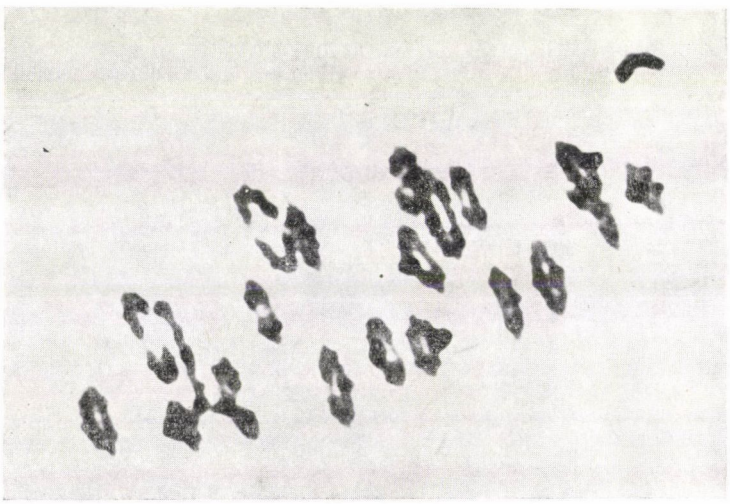

Fig. 3. Configuration of the metaphase chromosomes at meiosis in Rannyaya 12 mono-5A ($20^{II} + 1^{I}$)

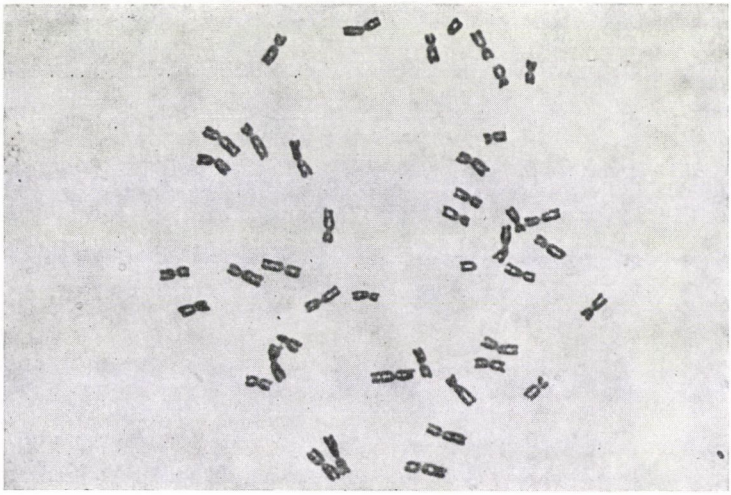

Fig. 4. Somatic chromosomes of a Chinese Spring ditelocentric line (two telocentric chromosomes are clearly recognisable)

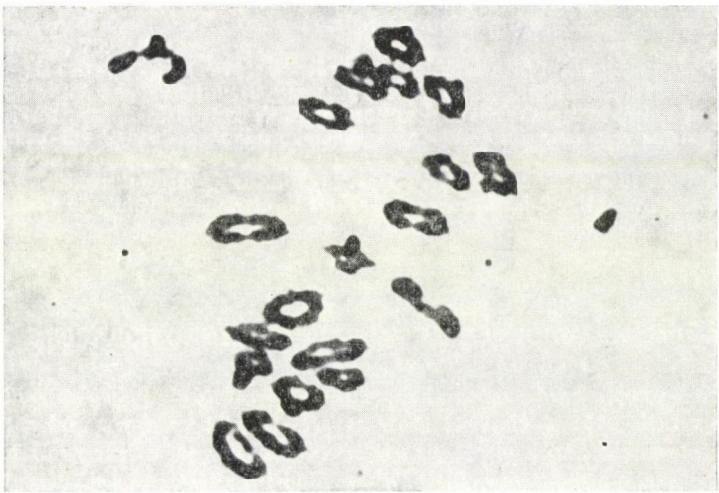

Fig. 5. Configuration of the metaphase chromosomes at meiosis in a monotelocentric plant (no univalent shift)

B variety. This back-cross is continued over 6—8 generations, then the monosomic line is maintained through self-pollination. The production of the monosomic line is complicated by the occurrence of univalent shift, the frequency of which may be as much as 30% depending on the variety (RÖBBELEN 1967—68, 1968, LAW—WORLAND 1972). To check the occurrence of univalent shift a test cross is carried out between the monosomes (Fig. 3) and the corresponding ditelosomes (Fig. 4). In the F_1 generation the meiosis of the monotelosomic plant is analysed. If, in metaphasis I, 20 bivalents and 1 telocentric univalent are found, then

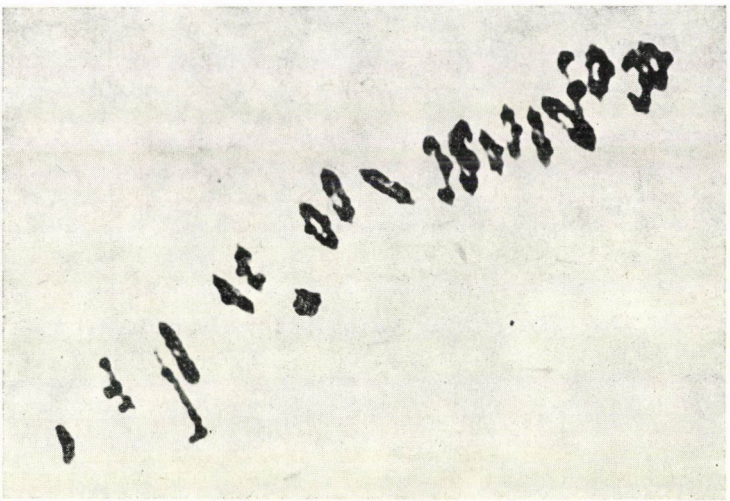

Fig. 6. Configuration of the metaphase chromosomes at meiosis in univalent shift

no univalent shift has occurred (Fig. 5); if, however, 19 bivalents, 1 heteromorphic bivalent and 1 normal univalent are found, then a different chromosome is missing from the given monosome than in the monosomic parent (Fig. 6). In developing a monosomic line complications may be caused by the presence of translocations between the wheat varieties (LAW—WORLAND 1972, BAIER *et al.* 1974, SUTKA 1978). In the meiosis of monosomic hybrids translocation brings about the appearance of a trivalent instead of a quadrivalent, which may be retained throughout the process of repeated back-crossing and makes the evolution of a true monosomic impossible.

Monosomic analysis

F_1 monosomic analysis is used for the localization of a recessive allele which appears in a hemizygous state. For this purpose the variety or mutant to be studied is crossed with each member of the monosomic set. In the F_1 generation the difference between monosomic and disomic plants is the basis on which the chromosome related with the given gene is spotted (MORRIS—SEARS 1967). If the disomic parent contains a dominant allele, or the recessive allele does not appear in a hemizygous state, so that no difference is found between the monosomes and disomes in the F_1 generation, then the monosomes and disomes must be selfed, and the localization of the given gene can be concluded on from the segregation ratio in the F_2 generation. In the F_2 of the monosome whose chromosome contains the critical gene the ratio of segregation differs greatly from the Mendelian ratio of segregation. It was with this method, for example, that the genes responsible for purple coleoptile in Mironovskaya 808 were localized at Martonvásár (SUTKA 1977).

With the help of telocentric chromosomes the distance and sequence of genes relative to the centromere, which is used as marker, can also be determined (SEARS 1962, 1966). Having established that gene Rc_3 is found in chromosome 7D let us examine in which arm and at what distance from the centromere this gene is to be found. To this end the Chinese Spring 7DS ditelocentric line, the coleoptile of which is green (rc_3), is crossed with the variety Mironovskaya 808 (Rc_3). The monotelodisomic plant obtained in the F_1 generation is crossed back to the variety Chinese Spring. Progenies with purple and green coleoptiles are cytologically

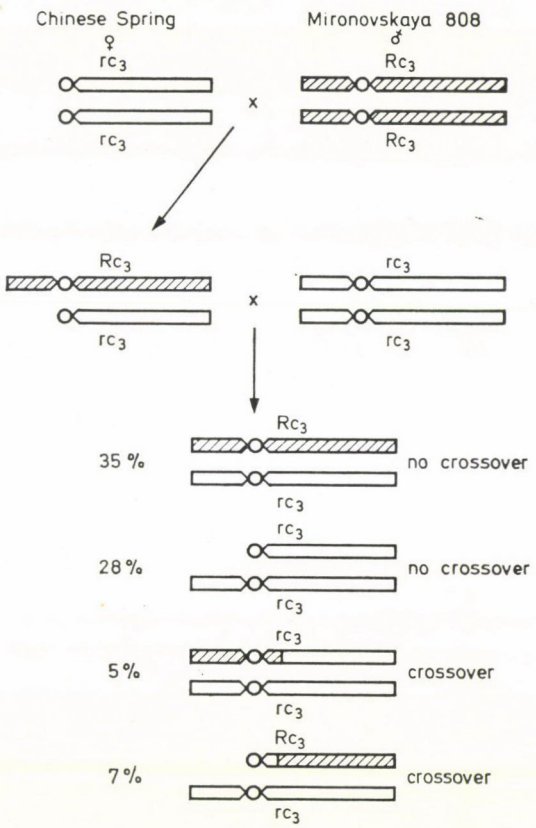

Fig. 7. Chromosome mapping (7D chromosome, Rc_3 — purple coleoptile)

checked for the presence of telocentric chromosomes (Fig. 7). Since there was a possibility of crossing-over in the heteromorphic bivalent, it can be determined from the frequency with which this occurs that the distance of the Rc_3 gene from the centromere is 16 ± 4.23 crossover units (SUTKA 1977).

By using monosomic analysis, relations with the individual chromosomes have been found in the case of many characters (KUSPIRA—UNRAU 1959, 1960, MORRISON 1960, TSUNEWAKI—JENKINS 1961, DRISCOLL—JENSEN 1963, 1964, MACER 1966, MAAN—LUCKEN 1966, SHEPHERD 1968, RAJKI—RAJKI 1969, HEAKEL 1970). Every year since 1959 Morris has presented a list of characters localized in various chromosomes in the "Wheat Newsletter". On the basis of these lists the data are sometimes ambiguous. This is due to the fact that in monosomic analysis univalent shifts and translocations, the frequency of which may modify the results depending on the wheat variety and the chromosome, are not usually taken into consideration. In the case of complicated quantitative physiological characters the allele differences are not fully displayed owing to the aneuploid state; the absence of chromosomes conceals the allele differences. This problem was encountered, for instance, in the monosomic F_2 analysis of frost hardiness in Mironovskaya 808 (SUTKA—RAJKI 1978). The errors in monosomic analysis can generally be eliminated with substitution lines and the localization of genes in the chromosomes can be made more accurate.

Inter-varietal chromosome substitutions

Chromosome substitution between varieties basically consists of replacing one or more chromosomes of the recipient variety by the chromosome(s) of the donor variety. This is justified for two purposes: 1. to study the effects of the individual chromosomes or genes in genotypes with different genetic backgrounds; 2. to improve the agronomical value of cultivated wheat varieties by incorporating a chromosome containing the required character.

The principles and methods for developing substitutions were first described by UNRAU *et al.* (1956). The primary and indispensable condition of each method is to have an aneuploid set available. In the case of the wheat variety Chinese Spring this precondition was satisfied by the early 1950s. The simplest method, in our opinion, is to cross the recipient CS monosome with the disomic donor, say variety B. In the F_1 generation disomic and monosomic forms are produced. The F_1 monosome is crossed back to the recipient monosome. This crossing of the two monosomes results in the segregation of monosomes, disomes and nullisomes. The monosome thus obtained is back-crossed six times to the monosomic recipient, then the monosome obtained in the last cross is selfed (Fig. 8). If the donor chromosome carries the recessive gene of an easily recognisable property as marker, then the presence of the desired donor chromosome will be phenotypically manifested. The substitution of 4B and 6B chromosomes of awned wheat in awnless varieties may be mentioned as an example. If there is no such marker, this method is less reliable, because it sometimes happens that at the end of the crossing programme the substitution line is either identical to the recipient variety, or, owing to univalent shift, it is not the desired chromosome that is substituted. Chromosome substitution is also complicated by reciprocal translocations between the varieties. The first difficulty can be overcome by selfing the selected monosomes in the F_1 generation, then in the back. cross generations, and crossing the recipient monosome back to the segregating disomes-

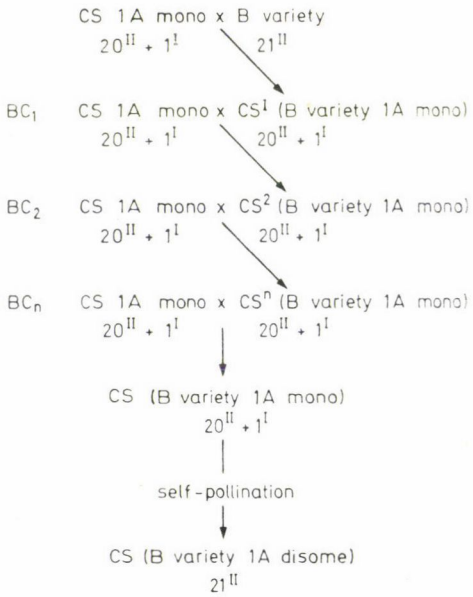

Fig. 8. Development of chromosome substitution using the recipient Chinese Spring monosomic set

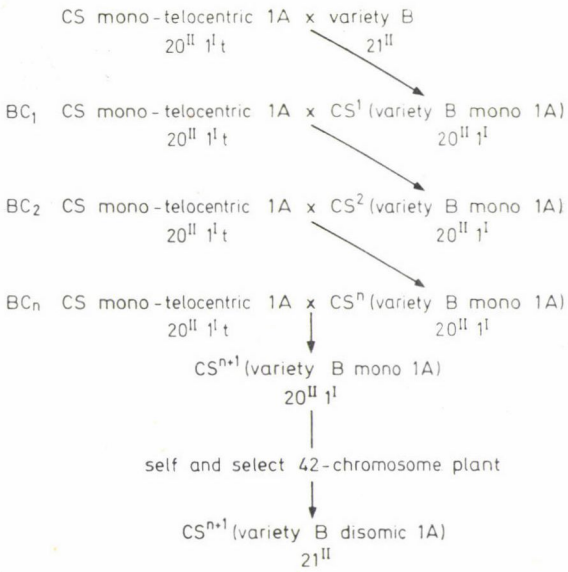

Fig. 9. Development of intervarietal substitution using a telocentric line. Chinese Spring is the recipient variety (after LAW—WORLAND 1972)

Unfortunately, with this method the substitution programme takes twice as long. The most precise method is to cross the disomic donor variety with a monotelosomic recipient (LAW—WORLAND 1972). In the F_1 generation and in the subsequent back-cross generations the monosome is selected and crossed back to the monotelosome (Fig. 9). The monosome obtained with the last back-cross is selfed, and the 42-chromosome plants are selected, in which a chromosome pair has thus come from the donor variety. This method has only been applied so far with Chinese Spring, since monotelosomic or ditelosomic lines are not available in other varieties. There are also other ideas for using telocentric chromosomes in producing intervarietal chromosome substitutions (MAAN *et al.* 1967, OKAMOTO—INOMATA 1976). In most places the method described first is used to bring about substitution, with the modification that the lines are produced at least in duplicate. This renders it possible to eliminate lines which contain errors.

The Chinese Spring substitution lines are primarily of theoretical importance. By comparing them with each other and with the donor and recipient an idea of how the substituted chromosomes influence the development of the given quantitative character can be obtained. Since 1966 Law has been carrying out detailed biometric analyses using chromosome substitutions in order to establish the number of genes controlling the quantitative characters, to map them, and to study the interactions between chromosomes (LAW 1966, 1967, 1972, LAW—WORLAND 1973).

Today the Chinese Spring substitutions are no longer of value for practical breeding. There is no point in substituting the chromosomes of the best winter wheat varieties into the recipient Chinese Spring variety, the latter still remains an extensive spring wheat variety. Substitution lines should thus be produced between intensive wheat varieties. The development of such substitutions can be started while the prospective variety is still under propagation, but the breeder already knows which of the characters will later be limiting and which of them needs improvement. Since the development of substitution can only be started with

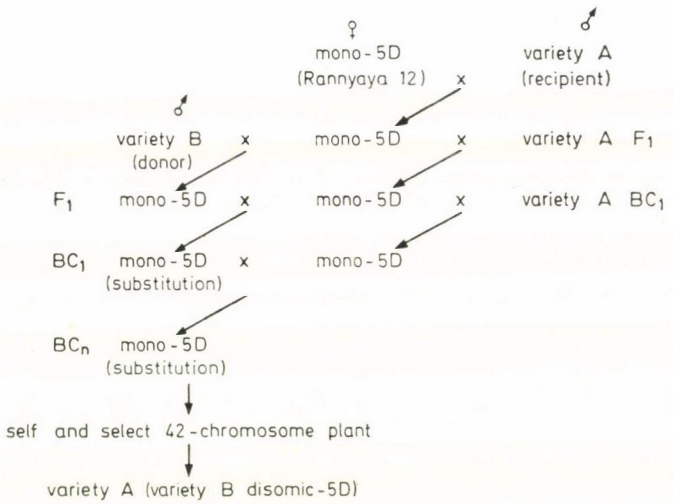

Fig. 10. Accelerated method for developing intervarietal chromosome substitution

a monosomic recipient, at least 12 generations are required for substitution between cultivated varieties. In order to shorten this rather lengthy process an "accelerated method" has been elaborated (SUTKA 1979). Let us assume that the protein content and frost hardiness of a prospective variety need improvement. From cytogenic analyses it is known that some of the genes controlling these properties are included in chromosome 5D (LAW—JENKINS 1970, KONZAK 1977, SUTKA—RAJKI 1978, MORRIS *et al.* 1978). The 5D chromosome of the variety with higher protein content and better frost resistance (donor) must thus be transferred into the prospective variety (recipient). According to the accelerated method the 5D monosomic line of an intensive wheat variety is crossed with the prospective variety or line. The F_1 monosomic plant is immediately crossed with the donor. At the end of the back-cross programme the 5D monosome of the prospective variety is obtained, and one generation later the substitution in which the 5D chromosome originates from the donor (Fig. 10).

Alien chromosome additions and substitutions

Modern plant breeding has been interested for decades in the variability of related species of cultivated cereals. These plants are usually resistant to diseases, more tolerant to severe cold, and the grain contains more protein than that of cultivated wheat. In the conventional crossing programme the value of an alien variation depends on whether pairing occurs between the chromosomes of the cultivated and wild species, in other words, whether there is any possibility of recombination. In most cases of interspecific and intergeneric crossing the genomes are so different that chromosome pairing and recombination will not occur. Cytogeneticists have succeeded in evolving methods by which the variability of alien species can be made use of in breeding. By these methods addition, substitution, translocation and homoeologous recombination are brought about.

Addition means changing the number of chromosomes by incorporating a chromosome or chromosomes from an alien species or genus into the full chromosome complement of the recipient. We speak of monosomic addition when a single chromosome is added to the full

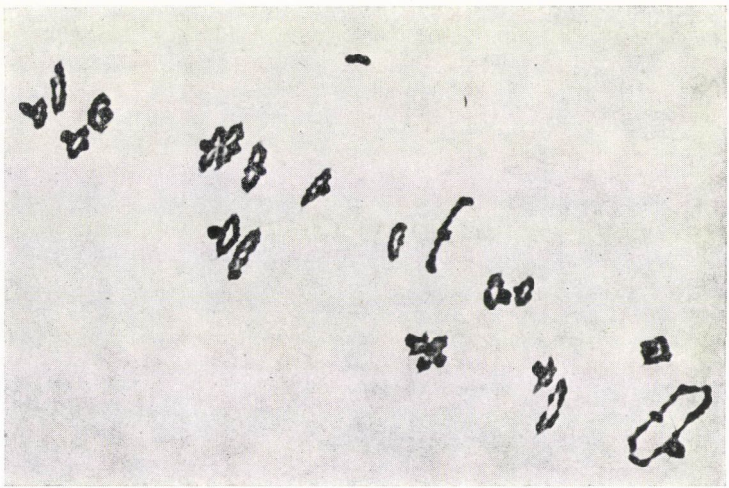

Fig. 11. First metaphase of meiosis in a monosomic addition ($1^{IV} + 19^{II} + 1^{I}$. Addition line courtesy of Dr. D. Szalay)

Fig. 12. Somatic chromosomes of alien disomic addition. (Single pair of *Agropyron* chromosomes added to the full complement of wheat chromosomes. 2n = 44. Addition line courtesy of Dr. D. Szalay)

chromosome complement (Fig. 11), and of disomic addition when a homologous chromosome pair is incorporated in the chromosome complement (Fig. 12).

The addition of a rye chromosome to common wheat was first discovered by LEIGHTY—TAYLOR (1924). This addition was known as hairy neck wheat. Systematic research work started only later, in the 1940s, when O'MARA (1940) began to produce artificial addition lines of wheat. The method consisted basically of backcrossing the amphidiploid obtained from *Triticum aestivum* × *Secale cereale* with wheat, and selecting from the progeny plants contain-

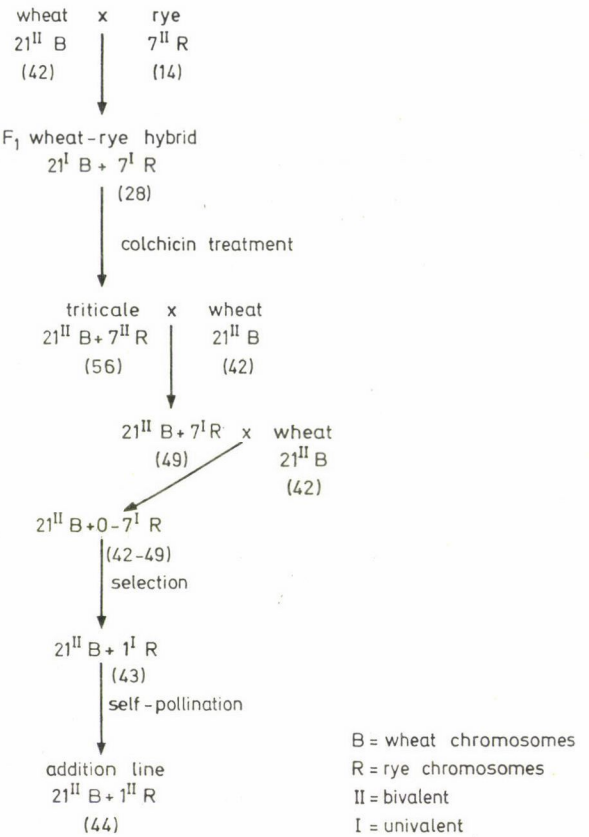

Fig. 13. Development of disomic addition

ing 42 wheat chromosomes and one rye chromosome each (Fig. 13). O'Mara was able to add 3 different rye chromosomes to wheat. Later EVANS—JENKINS (1960) brought about disomic addition for all seven rye chromosomes. With a slight modification of O'Mara's method SEARS (1956) added chromosomes to *Triticum aestivum* from *Aegilops umbellulata*, HYDE (1953) from *Haynaldia villosa*, RILEY *et al.* (1968) from *Aegilops comosa*, KNOTT (1961) from *Agropyron elongatum*, and WIENHUES (1971) and CAUDERON *et al.* (1973) from *Agropyron intermedium*.

The addition lines are superior to the recipient wheat in disease resistance, winter hardiness and in some morphological characters, but without constant cytological control the added chromosomes are easily lost. With respect to fertility and productivity they are mostly inferior to hexaploid wheat. The monosomic and disomic additions are thus of no immediate agronomical value, but they are important in producing substitution lines.

In the case of alien substitution a chromosome pair of the recipient species is replaced by a chromosome originating from a related species or genus. The first alien substitution was observed by KATTERMANN (1938) in wheat, where the 5A chromosome was substituted by the "hairy neck" chromosome of *Secale cereale*. A method for the artificial production of substitutions from alien species was described first by UNRAU *et al.* (1956) and then by RILEY—KIMBER (1966) (Fig. 14). In the first step a monosomic plant of the recipient species (wheat)

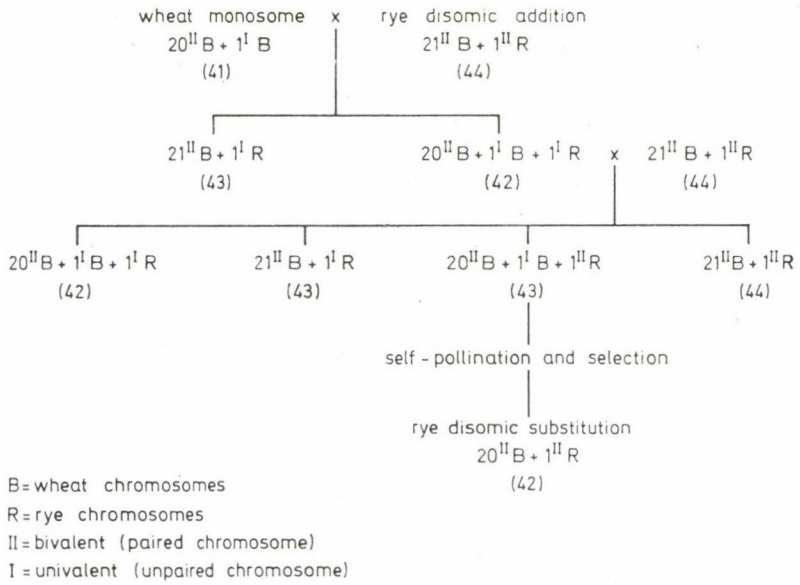

Fig. 14. **Development of alien disomic substitution**

is crossed with a disomic addition of the donor species (rye). Plants which are simultaneously monosomic with respect to the chromosome of the alien species and the specific chromosome of the recipient are selected from the progeny. These plants are either selfed, or recrossed with the disomic addition line. Plants containing 20^{II} wheat + 1^{II} rye chromosomes are selected from the progeny. If no distinction can be made between the alien chromosome and the other chromosomes, then the types of the progeny will be difficult to separate. To overcome this difficulty RILEY—KIMBER (1966) suggest the use of telocentric disomic additions from alien species.

In the case of an artificially produced alien substitution it is obviously known, which chromosome pair of the recipient species is substituted by the alien chromosome pair, while with a spontaneous substitution this must be identified. The fact that it is really a case of alien substitution can be determined by crossing the supposed substitution line with the euploid of the recipient species. The presence of n-1 bivalents and two univalents proves the existence of the alien substitution. In the opposite case n bivalents are found without any univalents. After that the alien substitution line is crossed with all possible monosomes of the recipient species, and metaphase I of the meiosis is analysed. The F_1 plants contain either n-2 bivalents and 3 univalents, or n-1 bivalents and one univalent. The latter configuration can only occur in the monosomic line in which the alien chromosome substitution took place. In this way KNOTT (1964) crossed a rust resistant alien wheat substitution with 21 monosomes of wheat. The alien chromosome pair originates from *Agropyron elongatum*. The $20^{II} + 1^{I}$ occurred only in the cross which included the 6A monosome, which thus suggests that the 6A chromosome of wheat was substituted by a chromosome pair of *Agropyron*.

The alien substitutions of wheat have so far come from the following donor species: *Secale cereale* (1R, 2R, 3R, 5R, 6R), *Secale montanum*, *Aegilops comosa* (2M), *Aegilops caudata* (1C), *Aegilops bicornis*, *Aegilops longissima*, *Aegilops umbellulata* ($1C^{u}$), *Aegilops variabilis*, *Agropyron elongatum* and *Agropyron intermedium* (ZELLER—FISCHBECK 1974, MAAN 1976, ZELLER 1976). The alien substitutions are usually cytologically stable. Some of them also have

normal fertility. Their success generally depends on the extent to which the donor chromosomes are able to replace the lost wheat chromosomes. It follows, that the so-called homoeologous, chromosomes genetically related to wheat, can be substituted more successfully.

An alien substitution may become a commercial variety in the following cases: 1. the meiotic stability is satisfactory, 2. the donor chromosome compensates for the missing wheat chromosome, 3. the alien substitution possesses a character, e.g. disease resistance, which is missing in the recipient species, 4. unfavourable, undesirable characters are not transferred with it to the recipient species.

These conditions are satisfied by the rust resistant wheat cultivar Weique. In this cultivar one wheat chromosome pair is substituted by a chromosome pair from *Agropyron intermedium*. In the wheat cultivars Zorba, Salzmünder Bartweizen, Orlando and Soladin the 1B wheat chromosome pair is substituted by the 1R rye chromosome pair. This substitution is also present in the breeding material of Riebesel 47/51, Weihenstephan 1007/53, St. 2153/63, Neuzucht and Wentzel (ZELLER—FISCHBECK (1974).

Substitutions have also been observed in hexaploid Triticale plants where rye chromosomes are replaced by chromosomes originating from the D genome of *Triticum aestivum* (GUSTAFSON—QUALSET 1974, LARTER *et al.* 1978). In the hexaploid *Triticale* cultivar Armadillo, for example, the rye chromosome 2R is substituted by the 2D wheat chromosome (GUSTAFSON—ZILLINSKY 1973). By means of the Giemsa chromosome staining technique it has recently been pointed out that in the course of *Triticale* breeding the telomeric heterochromatic regions may break off from some of the rye chromosomes and become eliminated. The meiotic chromosome pairing disorders and the cytological instability may thus be lessened (MERKER 1976, GUSTAFSON—BENNETT 1976).

Chromosome engineering

The term chromosome engineering means the process of chromosome segment transformation, during which a new chromosome is built up by mutagen treatment or by inducing homoeologous chromosome pairing. Above, the incorporation of a full chromosome from an alien species into the genome of a cultivated variety was described. The chromosome of an alien species usually carries undesirable genes too, although the breeder would like to transfer to the cultivated variety only a gene or genes controlling agronomically valuable characteristics. With this in view it is sometimes better to transfer only a segment of the alien chromosome.

The first successful segment transfer through translocation induced by X-rays was described by SEARS (1956), who transferred the leaf rust resistance gene of *Aegilops umbellulata* to the wheat chromosome (Fig. 15). In the first step *Triticum dicoccoides* was crossed with *Aegilops umbellulata*. Then the amphidiploid obtained by colchicin treatment was crossed twice with *Triticum aestivum*. The third step was to irradiate the monosomic addition line thus obtained, which contained the leaf rust resistant gene in the $6C^u$ isochromosome, immediately before meiosis. The *Triticum aestivum* flowers were pollinated with the irradiated pollen. By leaf rust testing and cytological examination 17 translocations were isolated from the resistant progeny; one of the translocation lines was rust resistant and at the same time did not contain unfavourable genes. This translocation line was named Transfer and was used as breeding material. Subsequent examinations revealed that the *Aegilops* segment was translocated into the terminal part of the long arm of the 6B chromosome of wheat (SEARS 1966).

For the incorporation of alien genetic material into wheat chromosomes DRISCOLL (1968) has elaborated a method which requires less cytological analysis. In this method the grains of a disomic addition line of wheat are irradiated. The M_1 plants are self-pollinated. The progeny

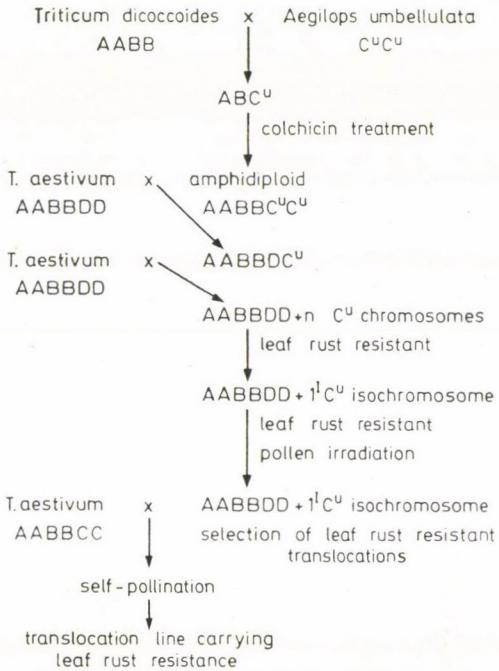

Fig. 15. Chromosome segment transfer from *Aegilops umbellulata* to *Triticum aestivum*

of one spike of each plant are sown in separate rows. The required translocation is selected on the basis of the segregation frequency of the desired property, e.g. disease resistance. It was in this way that a segment of the 2R chromosome of rye was translocated into the 4A wheat chromosome and the wheat cultivar Transec obtained, a wheat resistant to both leaf rust and powdery mildew. There are wheat cultivars (Benno, Feldhrone, Urban, Kavkaz, Avrora, etc.) in which a segment from the 1R chromosome of rye carrying a rust resistance gene was translocated into the 1B wheat chromosome in the course of breeding (ZELLER 1973, METTIN *et al.* 1973).

Induced homoeologous chromosome pairing was first applied by RILEY *et al.* (1968). The method consists in principle of suppressing the action of the asynaptic gene found in the 5B chromosome of wheat, which inhibits the pairing of homoeologous chromosomes. In their experiments the authors crossed the disomic addition of *Aegilops comosa* (2M) with *Aegilops speltoides*, which is able to suppress the action of 5B and thus make it possible for homoeologous chromosomes of wheat and *Aegilops comosa* to pair. The hybrids were backcrossed with common wheat. In the third generation a 42-chromosome yellow rust resistant form was selected which was heterozygous for the gene carrying the resistance and produced 21 bivalents in the meiosis. With the self-pollination of this form a homozygous yellow rust resistant wheat cultivar was obtained, which has become known by the name Compair. The subsequent cytological analyses revealed that the homoeologous recombination had taken place between the long arm of the 2M chromosome of *Aegilops comosa* and the right arm of the 2D chromosome of *Triticum aestivum* (RILEY *et al.* 1968).

SEARS (1967) suggests using an alien substitution to induce crossing-over between homoeologous chromosomes. In order to transfer the leaf rust resistance of *Agropyron elonga-*

Table 2

Chromosome segment transfer from related species to Triticum aestivum (after ZELLER—FISCHBECK *1974)*

Species	Chromosomes	Property	Variety (cultivar) or line	
Secale cereale	4A/5R	"hairy neck"	Chinese Spring	DRISCOLL—SEARS (1965)
	5B/5R	"hairy neck"	Chinese Spring	SEARS (1967)
	6D/5R	"hairy neck"	Chinese Spring	SEARS (1967)
	4A/2R	resistance to leaf rust and powdery mildew	Transec	DRISCOLL (1968)
	1B/1R	rust resistance	Weique "Translocation"	ZELLER—SASTROSUMARJO (1972)
	1B/1R	rust resistance	Benno, Feldkrone, Hamlet, Linos, Odilo, Perseus, Urban, Kavkaz, Avrora	ZELLER (1973); METTIN *et al.* (1973); BARTOS *et al.* (1973)
	4A/7R		Chinese Spring	ZELLER—KOLLER (1978)
	7B/4R		Chinese Spring	ZELLER—KOLLER (1978)
Aegilops umbellulata	9B/6C^{u}	leaf rust resistance	Transfer	SEARS (1956, 1961, 1966); ATHWAL—KIMBER (1972)
Aegilops comosa	2D/2M	yellow rust resistance	Compair	RILEY *et al.* (1968)
Agropyron elongatum	7D/7el$_1$	leaf rust resistance	Agatha	SHARMA—KNOTT (1966); KNOTT (1971); DVORÁK—KNOTT (1977)
	6A/?	stem rust resistance	P.W.327	KNOTT (1968)
	3D/?	leaf and stem rust resistance	Agent	SMITH *et al.* (1968); GOUGH—MERKLE (1971)
Agropyron intermedium	?	leaf and yellow rust resistance	Heine IV	WIENHUES (1967, 1973)

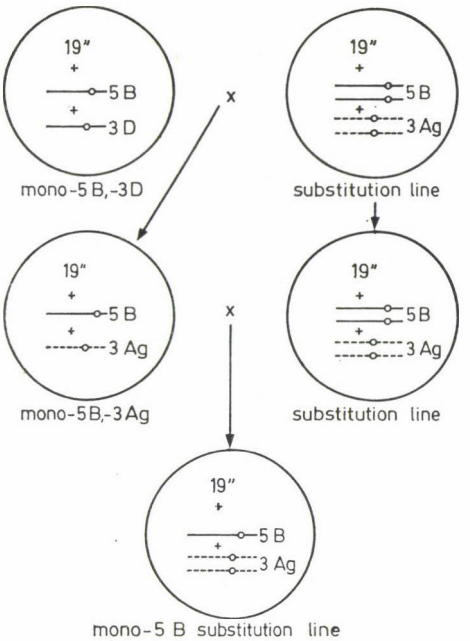

Fig. 16. Development of monosomic 5B *Agropyron* substitution (after SEARS 1972)

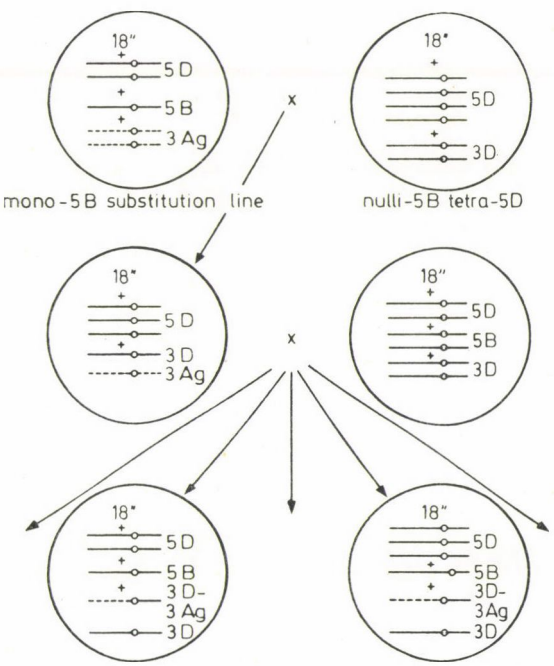

Fig. 17. Method for inducing homoeologous pairing (after SEARS 1972)

tum into the 3D chromosome of wheat he first transformed the alien substitution into a 5B monosome (Fig. 16), then crossed the substitution 5B monosome with the nulli-5B tetra-5D line (Fig. 17). In the hybrid the 3D and 3Ag chromosomes may have recombined owing to the absence of chromosome 5B. After being repeatedly backcrossed with an euploid this hybrid resulted in a leaf rust resistant wheat.

After ZELLER—FISCHBECK (1974), Table 2 presents cases of alien segments incorporated in *Triticum aestivum* chromosomes. It is to be hoped that this list will be extended in the future.

Plant protoplast fusions may offer new possibilities for chromosome engineering (BAJAJ 1974, MASCARENHAS *et al.* 1977). The production of somatic hybrids is a further possibility of fusing the genetic material of species and genera which cannot be crossed CARLSON *et al.* 1972, POWER *et al.* 1976, DUDITS *et al.* 1977). The practical application of cell and tissue culture techniques and of methods of producing protoplast fusions, so that, together with chromosome manipulations and chromosome engineering, they will serve the cause of wheat breeding, requires first and foremost the close co-operation of geneticists, physiologists, biometrists and breeders.

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*

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J. SUTKA

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AS I SEE IT...

J. LELLEY

FUTURE OF WHEAT IMPROVEMENT IN HUNGARY

I was requested by the editorial board to set forth my opinion on the future of wheat improvement in Hungary. I accepted the honourable task because the request was inspired by certain doubts raised by official statements, according to which the Hungarian improved varieties can no longer keep abreast in every respect with production requirements. Since the wheat varieties are also included in this accusation, it is time the situation was examined from the point of view of breeding to make it clear what has happened in past decades and what it is we have to reckon with in the future. I myself, as a breeder, took an active part in restarting the work of wheat breeding after the war, and shared in its problems and successes for 25 years. I trust that this lifetime experience will enable me to give a true picture of the situation and to outline future prospects.

It is a well known fact that up to the end of the fifties only domestic winter wheat cultivars were grown in Hungary, since in previous decades all attempts to introduce wheat varieties bred in Central or Western Europe failed. Such efforts met with failure even in large-scale farms with a highly intensive form of management. This long period of negative experience was thought to be due to the extreme continental climate of the Carpathian basin, combined with the relatively primitive methods of soil cultivation, fertilization and plant tending.

After such preliminaries it is understandable that the spectacular success of the short-strawed, large-spiked intensive type of new Italian winter wheat varieties on the Great Hungarian Plain in 1958—59 surprised growers and breeders alike. It was unexpected in spite

of the unprecedented yields of the winter wheat cultivars San Pastore, Fortunato and Produttore earlier reported from Yugoslavia. Most experts doubted that under the continental climatic conditions of the Hungarian Plain these varieties of Mediterranean origin would be competitive with the old steppe wheats with respect to winter hardiness and baking quality.

The wariness created by previous negative experiences was not unfounded. It soon turned out that in spite of their highly promising yield potentials the Italian winter wheat varieties could only be grown in the southern part of Hungary, owing to their deficient winter hardiness, and they were only suitable for feeding purposes because of their bad quality.

Nevertheless, the shock produced by the success of the Italian cultivars was not without its uses. The breeders realized that as a result of the rapid change-over to mechanized and chemized large-scale wheat production the steppe-type wheats were likely to become outdated overnight. It had been proved that short-strawed, large-spiked intensive varieties could be grown in Hungary provided their winter hardiness and baking quality could be improved by genetic intervention. This change in the breeders' view occurred at a time when their first post-war successes, the new winter wheat cultivars Fertődi 293, Kompolti 169 and Karcagi 522 were beginning to gain ground in commercial production. In spite of this, all the breeders were well aware of the need to set up entirely new objectives and to use a totally different initial stock.

The new programme had hardly been formulated and the preparations started when at the beginning of the sixties the Soviet-bred winter wheat cultivar Bezostaya 1 appeared, which combined in perfect harmony all the properties that the Hungarian breeders had just set up as their objective. This variety was found to be such a happy combination of all the properties required, from high productivity to good quality, and from winter hardiness to stalk firmness, that it swept the most promising Hungarian varieties off the wheat fields within a few years. In 1966—67 Bezostaya 1 was grown on some 80% of the total wheat growing area of the country. It should be added that soon afterwards, at the beginning of the seventies, the variety Bezostaya 1 was considered to be one of the best winter wheat cultivars in the world owing to its extraordinary adaptability, which was the result-of twenty years of very careful preparatory, crossing and selection work carried out by the Soviet academician P. P. Lukyanenko on the deep fertile layer of excellent quality soil on the Kuban Plain, in the same latitude as the southern part of the Hungarian Great Plain.

Thus the Italian varieties made the breeders realize that reorientation was necessary, while Bezostaya 1 provided the living example. However, in order to initiate an entirely new breeding programme, all the preparations, the collection of initial stock and the crossing had to be started again from scratch. Unfortunately even among professional plant growers there are few who realise that after such a complete change-over results of any importance cannot be expected for at least 8—10 years. The Hungarian breeders were no exception to this rule, the more so because by that time Bezostaya 1 had become a rival which proved almost unbeatable in variety trials all over the world. It was the shortness of the time that prevented Kiszombori 1, the first modest result of the new breeding programme, from becoming a real success. Although this cultivar was ready in 1963 it was not introduced into field production until 1968, after five years of testing, but it served to show how enthusiastically the breeders set to work to solve their new task.

The first prospective varieties to be really competitive with Bezostaya 1 were produced at the Martonvásár Agricultural Research Institute of the Hungarian Academy of Sciences in 1968—69, after exactly the length of time mentioned above. After that, new prospective cultivars equal to Bezostaya 1 in value followed in quick succession, not even giving ground to the recently introduced Soviet varieties Jubileinaya 50, Kavkaz and Avrora, which were grown in Hungary from the beginning of the seventies in proportions exceeding even Bezostaya 1. In 1973—74 nearly 90% of the total wheat sowing area in Hungary was occupied

Table 1

Yield average of commercially produced Soviet winter wheat varieties and new Hungarian prospective winter wheat varieties in the national small plot variety trials

Origin	1975		1976		1977	
	average		average		average	
	number of lines	q/ha	number of lines	q/ha	number of lines	q/ha
Soviet varieties	5	49.4	5	56.5	4	52.2
Best Hungarian prospective varieties	5	51.2	5	62.5	4	60.0

by Soviet varieties. However, in the national variety trials the situation had changed remarkably by then (Table 1).

The data in Table 1 clearly reflect the nature of this change. In 1975 16, and in 1976 and 1977 22 home bred varieties and prospective lines were included in the state trials, and the average yields of the best 5 of these in 1975 and 1976, and the best 4 in 1977, were substantially higher than those of the Soviet varieties. Although a comparison of the average yields does not reflect in detail the true situation, because occasional outstanding results of single strains remain hidden, it shows unmistakably the tendency of the change.

For the sake of simplicity only the grain yields are contained in the table. The varieties and lines were, however, competitive for other properties as well. That this was really the case is proved by Table 2. The data reveal that the situation has undergone a positive change since 1976. The target figures show that the confidence of the growers and administrators in Hungarian breeders has been restored.

Thus, the absolute dominance of the Soviet winter wheat cultivars lasted 10—12 years, not a year longer than was absolutely essential for the reorientation of breeding, for the production of the first reliable results, for the procedure of variety qualification and for the propagation of the seed of the new varieties. This by no means easy task was solved by the Hungarian wheat breeders in the shortest time possible. And if we consider that in the meantime all the wheat breeding establishments, with the exception of the Martonvásár Research Institute of the Hungarian Academy of Sciences, have been reorganized and relocated, so that

Table 2

Percentage share of winter wheat varieties of various origin in the 1,300,000 ha wheat sowing area of Hungary

Origin of the cultivated varieties	Percentage share of winter wheat varieties					
	Actual figure				Target figure	
	1973	1974	1975	1976	1977	1978
Hungary	3.0	2.3	6.3	13.6	35.0	43.0
Soviet Union	87.0	88.7	80.7	62.9	39.0	32.0
Yugoslavia	—	—	1.0	6.5	14.0	18.0
Italy	10.0	9.0	12.0	17.0	12.0	7.0

wheat breeding is no longer carried out at wheat breeding stations which were formerly of international repute, such as Bánkút, Kompolt, Fertőd, Sopronhorpács and Karcag, then this achievement deserves nothing but praise. This also explains why the first really competitive new winter wheat cultivars were produced at the Martonvásár Institute.

The data in Table 2 call attention to something else too. In spite of the spectacular success of the Hungarian varieties the sowing area of the Soviet varieties is still considerable. Italian varieties are also grown on about a hundred thousand hectares, though only for feeding purposes, while the sowing area of Yugoslav cultivare is currently increasing.

This fact proves that the earlier isolation of the Carpathian basin is finally at an end. This cannot be explained solely by the better and better varieties produced by the wheat breeders of the surrounding countries; the whole system of wheat production has fundamentally changed in the intervening period. The continual improvement in mechanical soil cultivation and the increasing volume of fertilizer application have almost completely eliminated the once so specific and decisive role of the edaphon. In industrial wheat production systems the soil has almost become an artificially uniform substrate. Its structure and nutrient composition is determined by the grower (Table 3). It is clear from the table that in Hungary the NPK

Table 3

Nutrients introduced with fertilizers and recovered by the wheat stand

Year	National average NPK consumption, kg/ha	National average yield on 1,300,000 ha, q/ha	Amount of NPK recovered in grain, straw, root, kg/ha	Percentage of NPK recovered, %
1975	250	32.0	206.4	76
1976	275	38.8	238.4	87
1977	280	40.6	240.5	89

content in the biomass of the wheat stand originates exclusively from the amount of NPK introduced by the growers into the soil substrate. We are thus witnesses of the uniformization of the edaphic factors which are of such great importance in the specificity of a region. And since the nurseries of every really successfully operated wheat breeding station are laid out on the best possible soil, it makes hardly any difference whether the breeding of a variety is carried out in Krasnodar, Mironovka, Zagreb, Novi Sad or Martonvásár. At the Krasnodar institute wheat breeding has been carried out on the best soil of the Kuban Plain for 60 years. At the Mironovka institute breeding has been in progress since 1945 on the best deep-layered Ukrainian soil. The Novi Sad institute has been operating for more than 25 years on the excellent soil of Bácska (between the Danube and Tisza rivers in Yugoslavia). Varieties originating from these places grow well in the abundantly fertilized fields of Hungary.

The effect of the new wheat production systems on climatic factors is not so decisive. In the Carpathian basin winter hardiness continues to be an indispensable requirement. That is why the production of Italian varieties in Hungary is risky. With the early varieties drought resistance, which was once so important, is now a less decisive factor. As a result of better soil cultivation practices and a balanced nutrient supply, the wheat plant utilizes the rainfall more economically. In the Krasnodar and Mironovka areas selection for winter hardiness or drought resistance is at least as successful as on the Hungarian Great Plain, nor is Novi Sad in a less advantageous position. This is why climatic conditions present no obstacle to wheat cultivars bred in these places. The situation is the same with regard to pathological resistance. Today, intensive breeding for resistance to leaf rust, stem rust or powdery mildew is carried

on at all wheat breeding sites. Efforts are being made to develop resistance to the widest possible range of pathogen races. This is why varieties bred in this way are strong rivals to the Hungarian wheat cultivars in the Hungarian state variety trials, as proved by the figures in Table 4.

Table 4

Comparison of the best early and medium early Hungarian and foreign winter wheat varieties for average yield on the basis of the results of national variety trials

Country of origin	Average yield					
	1975		1976		1977	
	number of lines	q/ha	number of lines	q/ha	number of lines	q/ha
Hungary	2	52.4	6	62.3	7	58.9
Yugoslavia	2	51.7	6	62.3	7	59.7
Romania	2	51.5	2	58.0	1	47.8
Bulgaria	2	51.5	2	57.9	4	55.0
Soviet Union	2	49.9	2	58.1	2	54.2

The data reveal that apart from Hungarian winter wheat varieties, Bulgarian, Romanian and Yugoslav varieties have become more and more competitive with the Soviet cultivars. Again, the summarized yield averages do not fully reflect the actual situation, because the averages may conceal the best combinations with outstanding productivity, but the tendency is obvious.

The increased keenness of the international competition, and evidence of the difficulty of the tasks to be solved are shown by Table 5, which summarizes the data of informative trials carried out by the Hungarian National Plant Variety Testing Institute in 1977.

It can be seen from the results that as regards potential productivity the Yugoslav and Bulgarian varieties, and even some Dutch derivates, were much better in 1977 than Bezostaya 1. Although again the comparison is not fully acceptable, it is nevertheless worth

Table 5

Average yield of varieties of various origin on the basis of national informative trials in 1977

Country of origin	Number of varieties included in the trial	Average yield, q/ha
Yugoslavia	14	61.9
Bulgaria	7	59.7
Holland	2	58.0
Italy	4	56.6
Romania	7	56.5
Soviet Union	5	55.5
Bezostaya 1 standard	(2)	52.9

noting that in the same year the average yield of the best 14 Hungarian varieties and lines was 56.6 q/ha in the national comparative variety trials, which are organized on the same scale. This result provides food for thought.

The total reorientation of Hungarian wheat breeding has been successful. The yielding potential of the new Hungarian varieties has substantially increased, though our neighbours have not been idle either, and they have utilized their excellent conditions to the maximum. Competition on this scale must, of course, be expected year in, year out, as this is a world phenomenon.

The data listed so far do not, however, give a full picture of the situation, as no information has been given on a very important property: baking quality. Outstanding yielding potential is undoubtedly very attractive from the grower's point of view. Nevertheless, in many cases growers do not insist on the introduction of certain foreign varieties, owing to their bad baking quality. At the same time there is a demand for varieties giving just as high yields combined with good baking qualities to be produced by Hungarian breeders. In this instance the statement made in the introduction, to the effect that Hungarian wheats cannot satisfy the almost exaggerated demands of the growers, seems to hold true.

From now on, breeders, growers and central administrators must continually reckon with the possibility that foreign varieties equal to or better than the Hungarian ones as regards productivity or even yield reliability will appear in the national variety trials or in the informative trials. There may be periods when foreign wheat varieties again occupy a certain proportion of the sowing area, but today this is a general phenomenon, especially in small countries.

Since N. Borlaug succeeded in producing daylength insensitive wheat varieties with the help of differences in daylength caused by a distance equivalent to 8 degrees of latitude (1,400 km) between the wheat nurseries of Ciudat Obregon and Toluca and by the 2,600 m difference in altitude, the geographical adaptability of wheat varieties is no longer confined to one continent. Since then, all over the world great attention has been paid to geographical adaptability when choosing crossing partners and carrying out selection. In the case of winter wheat, though, daylength insensitivity would be a very hazardous property, so this is not a practicable solution, but extensive variety and line trials carried out with international co-operation has led to the general improvement of geographical adaptability. This fact should not be under-estimated, but it would be a mistake to make the praise or criticism due to breeders and their prospects dependent on the zeniths or nadirs of the competition arising from this.

So far mention has been made almost exclusively of yielding potential, climatic and pathological resistance and geographical adaptability. It has been pointed out that in these fields the reorientation of wheat breeding in Hungary has been successful, opportunities have reached a balance, and there is no need to worry about the future of Hungarian wheat breeding. There is, however, another group of tasks which makes intensive wheat breeding extremely important in Hungary. This includes baking quality, total protein content and the biological value of wheat protein. Owing to our special ecological conditions and traditions we are in a better position in this respect than most of our potential rivals.

Hungary is expected to continue exporting wheat despite the fact that there is a lot of talk about growing grain fodders on a larger scale with a view to increasing meat exports. Excess wheat can only be sold at a good price if it has excellent baking quality. Naturally, good quality is not a matter of indifference from the point of view of domestic consumption either. In spite of the changing tendencies over the last few decades Hungarians still consume a large quantity of cereals. According to statistical surveys the annual per capita flour consumption is about 115 kg, the majority of which is wheat flour. With the present average total protein content of 12% this quantity represents nearly half the annual protein requirement

of the population, and almost 100% of the vegetable protein requirement. Every 1% increase in the total protein content of the approximately 16 million q domestic consumption would mean 160,000 q more protein without any extra input. If we were to succeed in improving the essential amino acid composition of the wheat protein (though the chances of this are not too good at the moment) the difference in biological value between wheat and animal proteins would be substantially reduced. The nutrition biological implications of such an achievement would be invaluable.

All three tasks are important, though they are not exclusively our responsibility. Nevertheless, owing to our traditions and ecological conditions they offer us a great possibility. In the international competition for productivity and yield reliability we have particularly good prospects in this field. Although quality improvement is also a major objective in the Ukraine, Canada and the United States, as far as Central Europe is concerned the task of producing wheat cultivars with outstanding quality falls mainly to Hungary.

In connection with the future of wheat breeding it may not be superfluous to speak of the prospects of the applicable methods. The Hungarian breeders are well aware of the fact that the more and more complicated tasks with which they are faced can only be solved with neocombinations derived from crosses prepared with great professional skill. Intervarietal crosses are likely to be the most successful. For a while interspecific and intergeneric hybridization will continue to be the method of the genetic laboratories. Induced mutation may also yield results, but because of its uncontrollable nature luck will continue to play an important role in it, so it will remain on the periphery of the breeding methods. It may perhaps produce unexpected results in breeding for quality improvement. Hybrid wheat research must be continued; the prospects are getting better and better, though it is unlikely to play an important role until the possibilities of cross breeding are exhausted, and we are still far from that. By the time this stage is reached it would be a good thing to clear up the unsettled questions on the extent and stability of the heterosis effect, the reliability of restoration and the practical production of hybrid seed. The reason why the prospects of cross breeding are still so favourable is than an increasing number of cultivars with high productivity become available to the breeders and this makes it possible to produce further neocombinations or transgressions. The frequently expressed opinion that the genus Triticum is deteriorating genetically has proved to be ill-grounded. On the contrary, in consequence of intensive genetic intervention all over the world a large number of new recombinations and mutations have been produced.

The relationship between genetic research on wheat and practical breeding should also be mentioned. There is no doubt that the genetic background of the Triticum genus has been analysed very thoroughly in the genetic laboratories for several decades. Today almost all the results of breeding are achieved thanks to the genetic knowledge originating from this basic research. It is therefore indispensable for practical breeders to keep abreast of the work being done in the genetic laboratories. At the same time it is a complete waste of time for breeders to carry on genetic research. It may occur that observations of theoretical genetic value are made during breeding, and it would be a mistake to miss such opportunities, but to breed a variety with the definite aim of carrying out genetic research usually proves to be abortive. Such efforts yield neither genetic nor serious breeding results. Basic research in wheat genetics is indispensable, but it requires a different type of professional training and knowledge than is necessary in practical breeding.

Thus, it is only possible to remain competitive if the breeder makes immediate use of the results of research in wheat genetics and physiology and applies the most up-to-date methods of selection which enable desirable neocombinations to be recognized as quickly and reliably as possible. Since research on breeding methodology and the elaboration of still more reliable, rapid methods and systems of examination is not the responsibility or interest of

anyone else, it falls to the breeders to fulfil this task. The successfulness of the decades to come will depend largely on the extent to which breeders succeed in improving selection methods. The more reliable the methods of selection are, the less the result will will be a matter of mere chance.

On these grounds there can be no doubt that Hungarian wheat breeders have been really successful. As the conditions in the nurseries are consolidated the prospects will become better and better. Keen international competition must always be expected in the future, but this will act as a stimulant. We have everything we need towards breeding wheat cultivars of outstanding quality, from favourable ecological conditions to long years of experience, so it would be a great mistake not to make use of this advantageous position.

J. LELLEY

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FORUM

OUR GUEST IS

SÁNDOR RAJKI

DIRECTOR OF THE AGRICULTURAL RESEARCH INSTITUTE
OF THE HUNGARIAN ACADEMY OF SCIENCES,
MARTONVÁSÁR

PÁL, GY.: *Dr Rajki, it is estimated that the world population will increase from 3.3 thousand million in 1960 to 6.4 thousand million in 2000, while according to UNFPA (United Nations Foundation for Population Activities) the number of unemployed is currently more than 300 million and will reach a thousand million by 2000. In your opinion is there any possibility of the forces of production developing fast enough to ensure an increase in general prosperity despite this vast increase in the population and the number of unemployed ?*

RAJKI, S.: On a world scale prosperity is a complex problem, though my qualifying it as such is not an attempt to avoid the issue. I could indeed say that as a geneticist and wheat breeder this is not my field, but as a thinking person I must and do have an opinion on this vital question.

The complexity of the problem lies in the fact that for the starving millions in the developing countries, for instance, the first step towards prosperity means avoiding starvation. And in the true sense of the word there is no "general" prosperity for society as a whole in the most highly developed capitalist countries, because the buying power of certain population groups is far less than that of others, and thus limits their ability to satisfy requirements which could in fact be met. Thus, prosperity is not general

even in these societies, but is differentiated to a greater or lesser extent from one population group to the other. But despite this, and despite the fact that the number of unemployed will rise proportionately with the ever increasing mechanisation and automation of simple work processes, the prospects for increasing general prosperity by the turn of the century are still rosiest in the most highly developed capitalist countries. The situation in these countries is also eased by the paradox that more food means less babies, which is a strictly human phenomenon, since both animals and plants respond to a higher rate of nutrition by producing more progeny.

The societies of the developing countries, which are fighting to overcome starvation, are plagued by the contrary of this paradox, namely that the lack of food is associated with a population explosion. North America can at times give food from its burdensome surplus or from other sources to keep starvation in check and to feed the hungry, but this is obviously only an interim solution. On the other hand, there are virtually no "starving countries" which show any signs of being able to help themselves within the foreseeable future, either with or without external technical aid or a green revolution.

The area of the world in which we Hungarians live is again a special case. Here, after achieving promising results under the influence of the initial impetus, the socio-economic limits to the development and utilisation of science and technology are becoming more and more apparent. Of these, mention should first be made of the theory and practice of neglecting or even restricting the development of the stronger and better and of concentrating solely on helping the weaker to catch up, and secondly of the fact that the efficiency level of the financial incentives and the degree of self-determination lag behind those offered by private ownership. These factors, together with the social depreciation of agriculture, are the fundamental reasons for the chronic shortage of food which has developed in this area of the world.

At present the restrictedly unrestricted arms race costs mankind an estimated thousand million dollars a day, and this burden is increasing perceptibly every year. In this arms race the maintenance of the alleged balance of power weighs more or less heavily on the opposing parties, depending on their economic potential. Mankind will wait in vain for an escape from this vicious circle on the basis of the old Scottish proverb "A wise man wavers . . .", or for any other reason dictated by common sense.

*

PÁL, GY.: *In Hungary 75.5 thousand million forints were invested in industry in 1977 and 25 thousand million forints in agriculture and forestry. In the same year industry produced 46.8% of the national income, while agriculture and forestry produced 17.7%. Imre Somogyi writes: "Our economic life should not be regarded and organised from the point of view of current politics, but from the perspective of thousands of years." In your opinion will investments of this order prove correct in the long run, in the distant future?*

RAJKI, S.: Food is the burning question of our age. In order to maintain any human activity whatsoever the prime necessity is to keep ourselves alive, and first and foremost this requires food, the quantity and quality of which is determined by plant production. There may well come a time when this latter will replace, at least to some extent, energy carriers of the unrenewable carbohydrate type as a source of energy.

Consequently, in our changing world, quite independent of the socio-economic system, human progress depends more and more on the achievement of an abundance of food. At present less than 10% of humanity lives in countries which can not only be

regarded as self-supporting with respect to food, but which also have a surplus at their disposal. For the present, the area of the world in which we Hungarians live is still obliged to pay thousands of millions of dollars a year for corn. As long as there is a lack of food anywhere in the world, food will be the hardest currency of all for those fighting the shortage and the strongest weapon in the hands of those who have a surplus. This situation demands that the development of agriculture and of the industries which serve the interests of agriculture, particularly the agricultural machinery and chemical industries and the food industry, together with the development of agricultural research and other relevant sciences, should be given the highest priority both in Hungary and elsewhere.

This complex development programme for Hungarian agriculture will be faced with even more imposing tasks if there is any truth in the prediction that in 50—60 years' time, i.e. by the end of the first third of the next century, we Hungarians will only have two resources at our disposal, one of which will be our wits and our bare hands, and the other the soil, because by then all our mineral wealth will be exhausted.

But whether this prediction comes true or not, our poverty in energy and industrial raw materials only serves to underline the need to give priority to complex agricultural development, since, directly or indirectly, the building blocks of food are essentially atmospheric carbon dioxide and nitrogen, minerals from the soil, and water from precipitation and the ground, while the energy is provided by sunshine. All of these are available plentifully, cost-free and in excellent quality in Hungary.

*

PÁL, GY.: *In Hungarian industry a considerable length of time is required for the introduction of a new product, the production of a prototype and the initiation of full-scale manufacturing, regardless of whether the product was developed in Hungary or is produced under foreign licence. A period of 13—14 months passes between the manufacture of the prototype and that of the first series. This is long enough for the product to lose its novelty, so that its price on the world market will drop. In agriculture how long do you think is required for the introduction of a new plant variety or a new cultivation technique ?*

RAJKI, S.: There is an inherent difference between industrial and agricultural products, such as a wheat variety, if for no other reason than that the latter is "organic", not "mechanical". This fundamentally determines the time required for the production and introduction of a new variety, and in this respect the comparison with industrial research and prototype production should be replaced by a comparison between rival plant breeders and seed producers as to who is faster and more efficient. The most important thing here, as it is everywhere, is inventiveness and foresight.

Continuing to take a wheat variety as our example, wheat research at Martonvásár is aimed at breeding a short-strawed (70—80 cm), high quality wheat with excellent resistance to pests and climatic adversities, capable of producing a reliable grain yield of 80—100 q/ha under farm conditions. It is only realistic to set this aim and to hope to achieve it in the foreseeable future if a phytotron is used to shorten the time required for breeding, to increase greatly the amount of breeding stock, particularly the number of new combinations which can be selected, and for the objective testing of the frost resistance of the varieties and lines. So first a phytotron had to be built and then we had to learn how to use it, and how to adapt wheat breeding methods to suit the phytotron.

As far as the introduction and seed production of new wheat varieties is concerned, the efficiency is considerably increased at Martonvásár by the fact that the

institute has its own experimental farm (naturally, this was not a gift from the gods either). The main task of this farm is to produce seed of new plant varieties as quickly as possible. But this is only one side of the coin. If a new plant variety is to be introduced rapidly, it is indispensable for the farmer, the state farm or cooperative who use the seed, to be financially interested in the development of production and the utilisation of new, modern techniques, as I mentioned in my reply to the first question. The significance of this factor cannot be overrated.

*

PÁL, GY.: *As a result of social development the increasing prosperity and the reduction in working hours has multiplied the number of people who have time, in addition to their work or after working hours, for other occupations, or hobbies. The majority of farm people work on their household plots, while many industrial workers garden as a hobby. Do you consider that the produce from these household plots and hobby gardens is significant from a national economic point of view, and do you think it likely that this source of produce will continue when the present owners, who are of peasant stock, are replaced by the up-and-coming generation ?*

RAJKI, S.: At present one sixth of the cultivated land in Hungary is taken up by household plots, subsidiary farms and small family holdings ranging from a quarter of a hectare to at most a few hectares. The size and importance of the production on these mini-farms depends on the current balance of power, and recently, far from decreasing, it has shown a welcome tendency to increase. Approximately half the pork and the majority of the eggs and of certain fruit and vegetables comes from the mini-farms. About half the produce of these mini-farms goes on the market.

The migration away from the smoke and noise of the concrete jungle is most pronounced in the most prosperous and urbanised societies. As a dwelling-place the sky-scraper is an anachronism. This attempt to go "back to nature" is becoming more and more general and in time it will no doubt conquer the whole of mankind. The family house with a garden has always been and always will be the height of our dreams. The increase in the number of garden and animal lovers shows a geometrical progression. Already the demand for land suitable for allotments exceeds the amount of land available. Steps should be taken to provide the necessary conditions before it is too late. Very wisely, an act was passed not long ago allowing the reacquisition by the original owners of the so-called closed gardens which were taken over by the state, but which are now lying fallow in many cases. We must just hope that the local authorities will be prevented from sabotaging this wise measure !

*

PÁL, GY.:*The steady improvement in agricultural conditions came to an abrupt halt in the last quarter of the 19th century and the situation immediately began to deteriorate. The reason for this was that the building of the railways and the work of land reclamation and river regulation were completed during this period, and tens of thousands of labourers who had been employed on these sites thronged back to the agricultural areas just at the time when conditions there also worsened. The price of wheat fell, wages were cut, buying power decreased and the number of unemployed rose. Do you think there is any likelihood of this*

situation repeating itself in our times once light and heavy industry has been fully developed ?

RAJKI, S.: In reply I can only mention a few of the means which might serve to paralyse the critical process described in the question.

a) The ideal in life for the women who currently make up a considerable proportion (apparently as much as half in some countries) of the total working population, is likely to change towards that of a wife and mother who works as a hobby in her spare time. At present the majority of wives and mothers work out of necessity, because the family needs the extra income. The hobby occupation of the future may be very varied, ranging from tending the family garden to acting as an outworker.

b) The possibilities offered by gardening are almost unlimited. The demand for freshly picked, ripe, red tomatoes, early peppers and other fresh vegetables is already great all the year round, independent of the season, and is increasing by leaps and bounds. What is needed is the establishment everywhere of large numbers of mini-greenhouses and polythene frames or tents, the latter being a modernised version of the traditional "hasura", which was woven from reeds or rushes and used in horticulture to cover hotbeds and greenhouses (Imre Somogyi). But this does not only apply to really fresh vegetables; the only way for freshly picked, ripe apricots and greengages and fragrant strawberries to reach the family table directly and unharmed is from small private gardens.

c) The reduction in working hours: a decade or two ago the 48-hour week was the battle cry of the workers, whereas now the German steel-workers in Westphalia are striking for a 35-hour week. This is a move in the right direction.

d) Instead of interminable speeches aimed at deluding the public into thinking that they are peacefully inclined, the governments should follow the example set by Costa Rica, who had the courage to demobilise the army and have consequently spent nothing at all on armaments for a considerable length of time !

*

PÁL, GY.: *In Hungary the average wheat yields increased from 20 q/ha in 1965 to 40.5 q/ha in 1977. In the same year, 1977, the yield average for extensive wheat growing in North America was 20.3 q/ha, while the average wheat yield in France was 44.5 q/ha, in West Germany 45.2 q/ha and in Great Britain 49.1 q/ha (on an area of more than 1 million hectares). Why do you think it is that the small farmers in capitalist countries where both industry and agriculture are highly developed, achieve higher wheat yields than the large socialist farms in Hungary ?*

RAJKI, S.: On analysing our wheat yields other people draw a completely opposite conclusion, namely that agriculture based on large farms is advantageous. They use growth dynamics in their arguments, i.e. the fact that over the last quarter of a century the Hungarian wheat yields have almost tripled, so that the approx. 40 q/ha yield average in Hungary may seem a more valuable achievement than England's world record of approx. 50 q/ha, as the latter only represents a one and a half times increase over the last quarter of a century. This is true enough, but using this argument France's peasant agriculture, which has also tripled its wheat yields during the last 25 years, would have a fair claim to Hungary's laurels.

Thus, the wheat yields achieved by large or small farms do not provide a proper basis for determining the optimum farm size. I am in full agreement with the opinion

of the excellent American farmer, David Garst, who claims that the key to farming efficiency is not size but careful management and the intensity of enlistment in the technological revolution, as I have already mentioned in my reply to the first question.

*

PÁL, GY.: *In liberal democratic states with a developed industry and agriculture the information put out by the vast, highly developed mass media serves to standardise people's opinions. The mass media are also used by scientific educationalists for the propagation of theories, views and concepts. Do you not think that the independent, individualistic thought required for scientific research is in danger of being standardised in Hungary too, due to the influence of the mass media ?*

RAJKI, S.: This problem is part of a more general one, namely the relationship between the quantity, the mass or the majority on the one hand, and the quality, the intellect or the minority on the other, and to a certain extent between the state and the individual. The consciousness of the vast majority is determined more or less by the mass media, and only a very small minority is capable or willing to examine critically the information issued by the mass media and to sieve and evaluate it. This thinking minority is recruited from practically all ranks of society. In other words, it includes not only writers and artists, scientists and teachers, but also peasants, labourers, etc. Their number would be far greater if the emphasis in education, both in the schools and elsewhere, were not on the cramming of ready-made knowledge, but on the development of the ability to think, and if minority, not to mention heretic, views were allowed to co-exist peacefully with the official views. There is and can be no creative science without the freedom to express and defend minority opinions . . . Rather than continuing to quote myself, let me instead quote just one sentence from an article by Gyula Illyés, published at Christmas, 1942: "The fate of our people, like that of all mankind, depends on whether we succeed in integrating the wishes of the masses with those of the intellect".

*

PÁL, GY.: *Confucius writes: "Truth does not deviate from human nature. If what is thought to be true deviates from human nature, it cannot be regarded as truth." The scholastics, however, consider that* "veritas est adaequatio rei et intellectus", *i.e. truth is the congruence of things and intellect. Do you think that in the field of sciences, and in the course of acquiring knowledge in this field, it is possible to speak of subjective and objective truths ?*

RAJKI, S.: In the sciences, which have become differentiated in modern times, cognition is not the same as intuition, since it is impossible to compare transcendent objects with knowledge independently of the knowledge itself, and the truth in general is not directly evident, but is based on proofs, i.e. it is indirectly evident.

Knowledge in which subjectivity dominates cannot be anything but false. Man tends to make judgments on the basis of his own experience, knowledge and prejudices, rather than on the proofs presented. Thus, a decision on the truth or fallacy of a new concept which does not depend on the individual and has a well-grounded claim to be regarded as an objective truth, is made in the light of the prevailing views. But to rearrange a well-known series of data, to judge it differently and to avoid the prevalent doctrine is the most difficult of intellectual acts. This is a practically invincible intellectual barrier, which pioneers such as Galilei were faced with, but which is to be found in more modest proportions beside the cradle of every original scientific discovery.

However discouraging, or even injurious, the resistance to new discoveries may be, it does have a certain value in that it protects science from the rash acceptance of ideas which are not sufficiently proved and tested. Nothing can cause greater damage to science than the abandoning of a critical standpoint and the easy acceptance of hypotheses supported by incomplete and half-tested proofs. N.B. A critical standpoint is by no means identical with scepticism!

*

PÁL, GY.: *According to Crick there are two general principles of protein synthesis, the sequence hypothesis and the central dogma. The central dogma was adopted by molecular genetics as the guiding principle of heredity. According to the central dogma information can only be transmitted in the directions DNA → protein and RNA → protein, but not from protein → protein, protein → RNA or protein → DNA. Since all the chemical reactions known so far are reversible, why, in your opinion, is the chemical reaction of information transmission not reversible?*

RAJKI, S.: Our opposition to Crick's dogma concerning the impossibility of transmitting genetic information in the reverse direction, which agrees in principle with Weismann's hypothesis denying, in its up-to-date formulation, the existence of adequate variations caused by environmental effects, is based primarily on experimental proofs, particularly on the fact that spring wheat can be autumnised, i.e. converted into winter wheat. The positive results of our autumnisation experiments, which were carried out in several cycles under field conditions, and thus cannot be reproduced with scientific accuracy, are well-known in scientific circles, both at home and abroad. The primary aim of this research is to give an answer to the cardinal question of biology, that of the heritability of acquired characters, but it is also hoped to find a method of bioregulation which will lead, provided it works under reproducible conditions in the phytotron, to the realisation of the geneticist's dream — the programmed alteration of heredity.

So far, the repeated use of a large number of phytotronic climatic programmes worked out by analysing the climatic conditions in the field during the autumnisation of classical and Mexican spring wheats has resulted in a maximum of two weeks' delay in the heading of the spring wheats examined. This is quite literally a half-success, because under Hungarian conditions a delay in heading of approximately a month is required before autumnisation can be recorded. The climatic programmes have been improved and further experiments are being carried out in order to achieve the phytotronic reproduction of the full process of autumnisation of spring into winter wheat as an adequate genetic variation.

The patent application entitled "Equipment for the investigation or optimation of the properties of organisms and/or methods for raising them", in which Martonvásár has a 50% interest, was granted in the United States of America in May 1978. Once this equipment has been manufactured and installed in our phytotron, it will be possible to use this super-modern optimation technique for genetic optimation in the form of autumnisation. A Canadian firm specialising in phytotronic equipment has been working on the manufacture of the new type of inhomogeneous phytotronic chamber for the last eighteen months, but even using the newest technology they do not expect to have the pilot unit ready for another year.

Nowadays, the characters of organisms and the effects and interactions of growing conditions are studied in equipment where discrete combinations of environmental factors (temperature, light, air humidity, nutrient solution, etc.) can be programmed for

a given period, thus creating a homogeneous environment. Using the equipment described in the patent our research aims can be achieved more simply, cheaply and quickly, using only a fraction of the experimental space, number of individuals and materials necessary for traditional methods. There is also the possibility of carrying out optimation processes which have seemed impossible so far.

With the help of a newly-acquired fourth-generation Hewlett—Packard computer, work has begun on the evaluation of a large number of climatic programmes to determine to what extent the various environmental factors included in the programmes contributed in the course of plant raising to the partial success represented by the two-week delay in the heading of experimental spring wheat plants. This will no doubt contribute to the perfection of the climatic programmes, which has already begun, and to complete success in achieving programmed autumnisation.

Every true geneticist is well aware of the theoretical significance of autumnisation. Winter habit, as an adequate genetic variation developing due to the effect of a changed environment, i.e. as the result of a modification in the metabolism corresponding to the environmental effect, and lacking in the initial spring wheat, is a case of the inheritance of acquired characters, i.e. of adequate variability. Since an adequate change corresponding to the direction and dimensions of the factor producing the change is brought about in the effect, the setting up of this scientific thesis is admissible, in accordance with the law of causality, even if some steps in the process are not clear, provided the end result is known.

The essential feature of Crick's central dogma, the impossibility of information transfer in the opposite direction, is *per se* undemonstrable, but according to the rules of logic its contrary is to be proved. In the same way the proof of the existence of the gene is a logical absurdity, because according to the classical and/or molecular gene concept this does not only signify the genetic material (the "DNA molecule") but also expresses the specific relationship between heredity and metabolism, the body and its environment, i.e. the impossibility of any adequate transcription of the information due to changes in the environment or the metabolism. Here too only the contrary can be proved. It is for this reason that the irrefutable proof of any adequate genetic variation, or, to quote Crick, the realisation of "any of the unknown transfers", would "shake the whole intellectual basis of molecular biology". Similarly, it is only "judicious" to discourse on the chemical preparation of a gene, i.e. a "DNA molecule" independent of changes in the environment or the metabolism, as long as no adequate variation has been irrefutably proved.

I was discussing this recently with a well-known New Zealand geneticist and one of the conclusions we reached reminded me of the views expressed by Ákos Pauler on science as a system of provable theses. Ákos Pauler writes that scientific theses form a "system, because there is a logical coherence between them all, since the determination of a new scientific thesis consists of fitting the new thesis into the existing system of theses. One of the criteria for the truth of a thesis is that it should logically fit in with the other true theses: if this is not so, either the new thesis or the old one, which is opposed to the new, must be modified or abandoned, for the simple reason that the prerequisite of science is the existence of a coherent system of truths."

The conclusion which can be drawn from autumnisation is too revolutionary: it is completely irreconcilable with the basic principle of current genetics, Crick's central dogma, which is a modernised version of Weismann's germ plasm hypothesis. Knowing the situation in genetics and the present-day possibility of proving the existence of adequate genetic variation, the resistance of the geneticists and their refusal to accept adequate genetic variation is self-evident.

From our viewpoint, the only possible reaction is to perfect the reproducible control experiments. As to your point about the reversibility of information transfer as a chemical (?) reaction, no doubt we can leave this to be answered by the competent people — the biochemists and the molecular geneticists.

*

PÁL, GY.: *Ever since Lamarck, or perhaps even earlier, the development of new hereditary characters in the plant organism due to the effect of the environment, and adequate to this environmental effect, i.e. the inheritance of acquired characters, has been the cardinal problem of genetics. As an apologist for the inheritance of characters acquired through the adequate modification of the metabolism, do you think that high yielding foreign wheat varieties of southern origin will become adapted to the Hungarian winter after a number of years of cultivation here ?*

RAJKI, S.: In my reply to the previous question I have already expressed my views on this subject, so I can confine myself to replying to the direct question. If by the adaptation of foreign wheat varieties of southern origin to the Hungarian winter during several years of cultivation here you simply mean the selection and propagation of the most hardy biotypes, closest to the winter type, already extant in the variety as a population, my answer is yes. But if by adaptation you mean the selection of biotypes more winter hardy and closer to the winter type than those originally present in southern varieties as a population due to the pressure of Hungarian winters, which are generally harder than those in their place of origin, my answer is no. Because if autumnisation were that simple the hypothesis concerning adequate genetic variations would have been proved long ago. This negative reply, I might add, conforms with the view expressed by Darwin, who disagrees with those who "imagine that natural selection induces variability". At the same time he stresses emphatically that selection "implies only the preservation of such variations as arise and are beneficial to the being under its conditions of life".

*

PÁL, GY.: *Thank you for your cooperation.*

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LECTIONES

NEGATIVE CORRELATION BETWEEN NPN RATIOS AND ENDOGENOUS CYTOKININ LEVELS IN MYCELIA AND LEAVES*

By isolating endogenous cytokinin-like biological activities (KIRÁLY *et al.* 1967 NGUYEN 1975) and applying synthetic purine-type cytokinins (POZSÁR 1967, POZSÁR *et al.* 1967) it has been directly proved that the protein nitrogen level (NPN) increases in the leaves relative to the dry matter and total nitrogen contents. The identification obtained by analysing the active group of synthetic cytokinins has provided a basis for interpreting the action mechanism of the side-effect of the cytokinins on protein synthesis (POZSÁR 1973). In the process of biological activity an important role can be attributed to the mechanism of proton activation and proton transfer with the changing ion type transformations (SZABÓ—POZSÁR 1974).

The endogenous cytokinin-like biological activity has been studied by means of numerous biological tests: chlorophyll preservation, stimulation of protein synthesis with the incorporation of labelled amino acids, increase of protein nitrogen level related to dry matter, stimulation of RNA and DNA synthesis, increase in the evaporation intensity measured with HTO, increase in dry matter content, increase in photosynthetic carbon dioxide ($^{14}CO_2$) fixation, etc., which were described in detail by NGUYEN (1975). Purine-type cytokinins were used in the tests as model compounds, since the analytical results obtained by SRIVASTAVA (1963) show that the cytokinin-like biological activities separated by chromatography are also purine-type compounds.

The results of comparative examinations, expressed as ppm values of the biological activity of benzyladenine in 10 g dry matter, are found in Table 1. The endogenous cytokinin-like biological activity tested by the incorporation of labelled amino acids can be regarded as 90% pure (NGUYEN 1975). The endogenous cytokinin-like biological activity isolated from the leaves of maize and cereals (wheat, barley) is remarkably low; the activity (level) of the fraction in the leaves of papilionaceous plants is 3—5 times higher. Furthermore, it is worth emphasizing that in the pathologically resistant (wild type) barley variety the endogenous cytokinin-like biological activity is significantly higher than in the susceptible barley lines. At the same time, the endogenous cytokinin level in the uredospores of phytopathogenic fungi hardly differs from the values obtained in the leaves of papilionaceous plants. However, the endogenous cytokinin-like biological activity isolated from infected leaves is nearly 10 times as high as that of healthy leaves, and almost identical with the cytokinin level measured in the reproductive bodies of the two examined species of *Basidiomycetes*.

According to the data in Table 1 there are differences of nearly an order of magnitude between the endogenous cytokinin-like biological activity measured in the leaves of mono-

* Lecture delivered at the Congress of the Hungarian Microbiological Association, Section on Agricultural and Industrial Microbiology held at the University of Forestry and Wood Industry, Sopron, on 17th June 1977.

Table 1

Endogenous cytokinin-like biological activity in 10 g dry matter, expressed in benzyladenine ppm. The biological activity was tested by incorporating labelled glycine —2-^{14}C the mean error of the mean value of the data is also indicated

Endogenous cytokinin-like biological activity	Cytokinin-like activity	
	benzyladenine in ppm	mean error of mean value
Maize leaf	7.1	0.52
Wheat leaf	8.6	0.70
Barley leaf, resistant	8.0	0.65
Barley leaf, susceptible	5.3	0.41
Bean leaf	31	2.6
Red clover leaf	72	5.6
Broad bean, root tip	53	4.1
Uromyces fabae, uredospore	43	3.6
Puccinia tritici, uredospore	37	2.9
Bean leaf, rust infected	518	46
Broad bean leaf, rust infected	467	38
Coprinus micaceus, reproductive body	652	48
Agaricus bisporis, reproductive body	652	51

cotyledonous and papilionaceous plants and that found in mycelium-infected leaves and reproductive bodies.

In our experiments the NPN ratio, expressed as a percentage of the total nitrogen level shows a negative correlation with the endogenous cytokinin-like biological activity, since the lower cytokinin level is accompanied by a higher NPN ratio, and the higher cytokinin level by a lower NPN content, as seen in Table 2. The NPN represents primarily the free

Table 2

NPN content in leaves of plants and reproductive bodies of basidial fungi, expressed as a percentage of the total nitrogen content, (The mean error of the mean value does not exceed 8%)

Samples	NPN as percentage of total nitrogen
Maize leaf	51.2
Wheat leaf	45.6
Bean leaf	31.4
Broad bean leaf	28.5
Coprinus micaceus reproductive body	7.3
Agaricus bisporus reproductive body	6.1

amino acid content, together with the amine, amide, nitrate, nitrite and ammonium levels. In the plant samples the free urea content is negligible. In fungi the non-essential free amino acids and amino acid derivatives occur in greater diversity than in green plants.

The negative correlation between the endogenous cytokinin-like biological activity and the NPN levels directly demonstrates that the cytokinins play a role in controlling protein nitrogen levels and ratios in the leaves of higher green plants and in producing the very high protein nitrogen content of mycelia.

Besides its importance in plant breeding, the phenomenon is also significant for production biology due to the protein nitrogen levels.

The outstandingly high endogenous cytokinin-like biological activity measured in reproductive bodies was reported in an earlier publication (SZABÓ *et al.* 1970), where it was demonstrated that when the endogenous cytokinin-like biological activity is added to synthetic cytokinins the result may even be an additive effect; this is particularly remarkable because the respective purine derivatives were previously considered to be genus specific. The additive effect (NGUYEN 1975) can also be regarded as important from a theoretical point of view. At the same time the total nitrogen content is considerably influenced by a number of plant hormones and antimetabolites, as indicated in an earlier paper (SZABÓ *et al.* 1972).

T. SZARVAS, B. I. POZSÁR

Institute of Isotopes
of the Hungarian Academy of Sciences
1525 Budapest
P.O.B. 77
Konkoly-Thege út

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EXPERIMENTS TO INCREASE PROLIFICACY IN ROMANOV SHEEP*

For the last twenty years many accounts of the results of intensive sheep breeding have been published. When estimating the possibilities of a biological increase in wool, milk and meat production and calculating the rise in costs involved with the expected yield increase at world market prices FÁI (1974) arrived at the conclusion that it was an improvement in the meat-producing capacity of the sheep that promised the largest income. The biological reserves hidden in the breed, and the increasing world market demand for mutton also favour the development of intensive mutton production. In this field England took the initiative by developing new highly prolific breeds, such as Colbreed and Cambridge, which are capable of giving birth to and raising three lambs at a time. Three lambs per ewe per year are also guaranteed in sheep crossed with the paternal lines Cobb 101 and Cadzow improver. LAND—McCLELLAND (1971) aimed to obtain lambs twice a year, but for the time being this did not seem feasible owing to the inhibitory effect of suckling on mating and conception. ROBINSON *et al.* (1976) reported on the repeated lambing every seven months of Finnish-Dorset; using hormone treatments to induce heat they obtained an average of 3.45 lambs per ewe per year.

On the experimental farm of the Agricultural College, Kaposvár, a series of model experiments has been set up in order to utilize the reproduction biological potential of sheep to the maximum, and thus to obtain a larger meat yield primarily due to an increased rate of lambing.

Our experiments were based on the following hypothesis:

- with higher prolificacy more lambs can be obtained from fewer ewes,
- young rams and ewe lambs raised for breeding purposes can be reproduced with a lower number of ewes,
- a larger proportion of the ewes can be crossed with meat-type rams
- cross-bred slaughter lambs can be produced with heavier weights, with a higher meat output and with better carcass quality,
- in a stock of ewes used as maternal partners in crossing, selection objectives other than early maturity, fertility and prolificacy can be neglected.

A small number of Finnish ewes and rams were imported from Finland and a similar number of Romanov sheep from the Soviet Union and these were kept in the same flock under identical feeding conditions so as to compare their qualities.

The results of our investigations have already been published (VERESS *et al.* 1975, VERESS *et al.* 1976a, 1976b). On comparing the two breeds mentioned similar results were obtained in France (RICORDEAU *et al.* 1976) and in Czechoslovakia (JAKUBEC—KRIZEK 1975). The Romanov breed has been found superior with respect to acclimatization, suitability for being kept in large flocks, non-seasonal oestrus and also prolificacy. Our report is therefore limited to the results obtained in the course of an experiment with Romanov sheep.

The data presented subsequently were collected from the spring of 1973 to the spring of 1977 on imported animals and their pure-bred progenies. In one case information is given on sexual maturity in the progenies of Finnish ewes mated with Romanov rams.

The animals were purchased in the autumns of 1973 and 1974. The experimental sheep-barn, where the conditions were up to the required standard, was not completed until the spring of 1976. Until then the animals were kept under conditions which were far from satisfactory.

Our aim was to increase the stock of ewes, so every female lamb was retained for breeding purposes, except for incurably ill or infertile ewes. This meant that 10—15% of the ewes

* Lecture delivered at an international conference on genetics organized in Vyskov, Czechoslovakia, between 11 and 13 October 1977.

were discarded each year. Lambs from larger litters were bottle-fed with milk substitutes up to the spring of 1976, but this method was far from satisfying the requirements for the up-to-date raising of large litters.

To start with, the sheep were mated every three months, which meant that lambing also took place at three-monthly intervals, with a view to collecting data on the animals' readiness for rutting and conception. Heat induction by hormone treatments was not employed. Since the autumn of 1975 we have changed over to mating in autumn (August-September) and in spring (April-May); accordingly lambing now takes place only twice a year.

The stock was grazed all the year round, when possible even in winter. Alfalfa hay, pea-straw, maize silage and mixed feeds consisting mainly of rye were fed as feed supplements. The suckling period of newly-dropped lambs lasted 30—40 days. Before and after weaning, up to the age of 100 days, they were given a granulated feed mix of our own composition (VERESS—KAKUK 1971), which satisfied the nutritional requirements of the lambs.

Our own investigations cover three areas:

— possibilities of earlier use in breeding,
— higher number of viable progeny per lambing,
— more frequent lambing.

In Romanov lambs sexual maturity can be expected after the age of 150 days. Romanov lambs dropped in the springs of 1971 and 1972 were found by THIMONIER (1975) to come into heat for the first time between 158 and 243 days of age. The time of the first heat showed less variation than the date of birth. Earlier sexual maturity may be related with the shortening autumn days. In the autumn of 1976, when the standard of the conditions under which the stock was kept became satisfactory, the 16 Romanov and Romanov×Finnish F_1 ewe lambs kept for breeding purposes developed and grew more favourably; seven of them (44%) became sexually mature between 17 December 1976 and 27 January 1977, at an average age of 130 days (maximum 142, minimum 109 days); their average live weight was found to be 21.9 kg, the extreme values being 26.2 and 19.0 kg.

GAÁL (1957) notes that in 1933 properly fed Hungarian merino ewes lambed successfully at the age of one year. A slight ($r = +0.2$) but positive correlation between the age of lambing and the number of progeny has been found, but this cannot be regarded as reliable because of the great individual variation. The lambing age and number of progeny of the 79 ewes in first lamb listed in Table 1 ranged between wide limits, since we tried to mate all

Table 1

Time of first lambing and size of litter

	n = 79		
	$\bar{x}$	s	v%
Age at first lambing (days)	525.09	150.50	28.70
Average litter size	2.34	0.83	35.46

the ewe lambs considered suitable for breeding, even the underdeveloped ones, in order to increase the stock. The number of progeny and the average number of lambs fit for weaning only showed an increase until the age of 22 months (Table 2). It must be supposed that the group of ewes older than that consisted of underdeveloped individuals, which rutted later, became pregnant less frequently, dropped fewer lambs and raised them less satisfactorily. Thus, provided the animals are raised under suitable conditions, first lambing between 12 and

Table 2

Effect of age at first mating on the rate of lambing and raising

Age at first lambing (days)	Number of sheep		Mean lambing age (days) $\bar{x}$	Prolificacy %	Weaning, %
	n	%			
≤ 420	15	19.0	373	187	113
421—480	19	24.0	457	211	121
481—540	29	37.0	517	259	200
541—600	3	3.7	553	267	233
601—660	2	2.5	648	300	300
661—720	3	3.7	707	267	100
≥ 721	8	10.1	893	250	150
Total or mean	79	100.0	525	234	159

18 months of age is not only desirable from a biological point of view, but is also considerably more economic.

Many specialists have reservations on the subject of breeding prolific breeds for meat. Among others things they raise the objection that the mortality of lambs from larger litters is so high that it is doubtful whether selection for prolificacy is economical.

As shown by the data in Table 3 the average number of progeny is 2.57, the number of live births 2.12 and the number of lambs raised 1.82. Compared to the data for intensive sheep keeping these results are by no means satisfactory, though the average is considerably lowered by the fact that 40% of the lambings evaluated were first lambings, and selection for larger litters was not possible at this stage. No disadvantageous correlation could be revealed between the size of the litter and the proportion of lambs reaching maturity. The data prove that under good feeding conditions Romanov ewes are capable of producing four or five lambs at a time without any considerable decrease in the extrauterinal viability of the lambs. The birth weight, on the other hand, is of decisive importance from the point of view of survival (Fig. 1). According to our observations, the death of lambs with a birth weight

Table 3

Trends of litter size, viability and raising percentage

Litter size	Lambings		Born, n	Born alive, %	Raised, %
	n	%			
Single	29	14.7	29	82.7	69.0
Twins	72	36.5	144	85.4	73.2
Triplets	59	30.0	177	83.6	74.0
Quadruplets	28	14.2	112	80.3	67.9
Quintuplets	9	4.6	45	71.7	60.0
Σ or $\bar{x}$	197	100.0	507	82.2	70.6

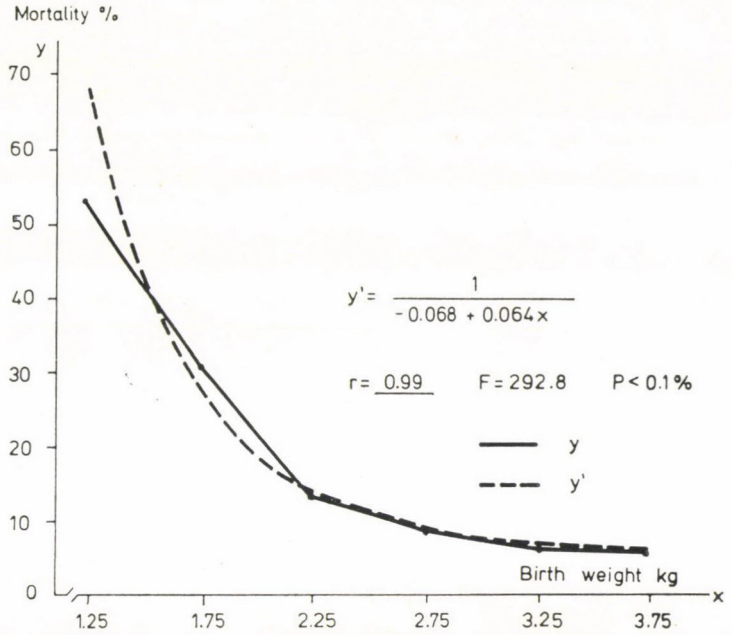

Fig. 1. Correlation between birth weight and rate of mortality

of around 2 kg cannot be explained by reduced viability; the conditions under which the animals are kept must be held responsible.

It is inadvisable to raise the lambs of a high prolificacy stock exclusively by suckling. The gestation of 4—5 lambs, even under optimum feeding conditions, overburdens the organism of the animal. Considering 1 litre milk as the optimum daily requirement for a lamb, the organism of a ewe weighing about 50 kg is hardly suitable, after the exhausting pregnancy, for the transformation of the surplus energy necessary to produce the total amount of milk indispensable for 4—5 lambs. Therefore, last year, if there were no complications during lambing and the ewes were in good physical condition, no more than two lambs were allowed to suckle each ewe, the rest being fed with an automatic suckling system designed by BURGKART (1973).

The daily weight gain of lambs weaned at the age of 1—2 days and raised artificially until 28 days of age showed a better daily weight gain than that of lambs from the same litter

Table 4

Daily weight gains of suckled and artificially raised lambs till the age of 28 days

Raising method	Number of lambs	Daily weight gain (g)			$\bar{d}$	t	P%
		$\bar{x}$	s	v%			
Suckled	28	164.83	45.99	27.90	32.56	2.93	<1
Rised using a suckling machine	32	197.39	40.16	20.34			

raised by the ewe (Table 4). Lambs born with a relatively low weight, which were raised artificially, could suck at any time according to their requirements, and their supply with milk substitutes was also adjusted to give an optimum rate of growth. The elaboration of a reliable technology for artificial raising is therefore considered an indispensable basis for raising the lambs of high prolificacy breeds without losses and taking advantage of their genetic capacity.

The correlation between the number of lambings and the size of the litter was also examined (Table 5). The number of progeny was found to increase moderately with the number of lambings. The question of whether an increase in the size of the litter influences the time between the successive lambings was also considered. The data in Table 6 show that the interval between two lambings slightly increased for ewes producing larger litters. Nevertheless the variation between the individual cases and the results of variance analysis both suggest that conditions other than the size of the litter have the greatest influence on the reproduction of the ewes.

Table 5

Number of lambings and ratio of lambs born and raised

Lambing	n	Litter size per lambing, $\bar{x}$	At the age of 28 days, $\bar{x}$	Proportion raised, %
First	79	2.34	1.60	68.1
Second	53	2.45	1.68	68.5
Third	32	2.75	2.28	83.0
Fourth	20	3.20	2.00	62.5
Fifth	10	3.10	2.20	71.0
Sixth	3	3.00	2.67	88.9
Σ or $\bar{x}$	197	2.57	1.82	70.6

Table 6

Litter size and lambing interval

Litter	Number of lambings n	Lambing interval		
		$\bar{x}$	s	v%
Single	18	244.06	57.45	23.54
Twins	47	256.72	68.76	26.78
Triplets	27	253.10	83.23	32.88
Quadruplets	17	286.82	93.45	32.58
Quintuplets	2	300.00	108.89	36.29
Σ or $\bar{x}$	111	259.19	75.57	29.16

Variance analysis

Factors	SQ	FG	MQ	F
Total	627,008.05	110	—	—
Treatment	21,722.85	4	5,430.71	0.95
Error	605,285.20	106	5,710.24	—

Table 7

Lambing interval, and ratio of lambs born and raised

Categories (days)	Lambings		Average time of subsequent lambing (days)	Litter size			Weaned, n	Raised, %
	n	%		$\bar{x}$	s	v%		
≤ 180	8	6.7	173.9	1.75	0.71	40.41	1.25	71.4
181—210	31	26.1	197.5	2.68	1.25	46.64	1.97	73.5
211—240	18	15.1	223.4	2.78	0.65	23.28	1.89	68.0
241—270	18	15.1	254.4	2.89	1.27	44.25	1.94	67.0
271—300	12	10.1	285.3	2.83	1.34	47.19	2.33	82.3
≥ 301	32	26.9	401.2	2.94	1.22	41.41	2.09	71.7

In investigations with merino sheep (VERESS—VÉGH 1974) the number of progeny has been found to be substantially reduced when lambing takes place every 8 months. In the case of Romanov ewes a substantial decrease in the number of progeny was ascertained for ewes rebred in a period shorter then 180 days. If the period was longer than this, it had practically no influence on the size of the litter.

The time between two successive lambings was 259 days on average, though no selection has been made for this character (Table 6). On average the frequency of lambing was 1.41 per ewe per year. This figure proves that rutting is highly independent of the season in this breed. About 33% of the ewes conceived within 54 days after lambing. Although the time between successive lambings decreased with an increase in the number of lambings (Table 8), the

Table 8

Lambing interval (days) as a function of the number of lambings

Lambing	n	Lambing interval (days)		
		$\bar{x}$	s	v%
First		285.39	130.22	45.63
Second	53	260.25	78.29	30.08
Third	32	267.97	76.56	28.57
Fourth	20	232.20	60.65	26.12
Fifth	10			

Variance analysis

Factors	SQ	FG	MQ	F
Total	1,246,150.17	114	—	—
Treatment	29,914.94	3	9,971.65	0.91
Error	1,216,235.23	111	10,957.07	

individual differences were so great that no statistical correlation could be established for this phenomenon.

The number of lambs dropped per ewe was 3.62 a year, of which 2.97 survived and 2.56 were weaned. The excellent constitution of the Romanov breed is indicated by the fact that 3 of the ewes lambed for the sixth time before reaching the age of 5 years. KOVNEREV (1969) reported on Romanov ewes kept in breeding up to the age of 10 years; they lambed 12—16 times and produced an average of 28.8 lambs per ewe.

It cannot, however, be stated that Romanov sheep are equally inclined to rut in all seasons of the year. While the merino is least likely to be in heat in the months of March and April, the least disposition for rutting in Romanov sheep was observed by KOVNEREV (1969) in June.

In the test stock the time of mating after weaning was divided into two different periods, according to increasing and decreasing daylength. As shown by the data (Table 9),

Table 9

Effect of season on conception and prolificacy

	From 21 June to 20 December	From 21 December to 20 June
No. of ewes	83	93
Mated	71	62
Rutting percentage	85.5	66.7
Lambed	50	34
Lambing percentage	60.2	36.6
Number of lambs born	139	85
Rate of prolificacy	278	250
Number of lambs weaned	105	63
Percentage raised	76.0	74.0

decreasing light influenced not only the induction of oestrus but also the rate of conception and the number of progeny. A statistically proved, well perceptible difference was observed between the two illumination periods, which again calls attention to the importance of this insufficiently emphasized but fundamental factor of the environment.

Our investigations indicate that the acclimatization and reproduction abilities of the Romanov breed make it suitable for the development of a maternal population in which

selection for early maturity, high prolificacy and frequent lambing can be made simultaneously. The individual variation obtained for these characters was so high that success in this threefold selection seems probable. High prolificacy also holds promise of greater selection stress.

Barn keeping and intensive feeding all the year round are not considered necessary even when high prolificacy is aimed at. Modern grass management makes it possible to increase the health and resistance of the animals by grazing and by the movement thus involved. Costs can be reduced if the sheep satisfy their feeding requirements directly during the grazing season, thereby rendering the use of expensive harvesting machines superfluous.

Precisely elaborated technologies are required for all phases of breeding and raising. High productivity animals are particularly sensitive to feeding conditions.

Romanov ewes crossed with meat-type rams may also serve for the production of large volumes of high quality mutton.

The authors are convinced that the realization of the objectives outlined above would considerably increase the profitability of sheep breeding, enabling it to be included among the intensive production branches of agriculture.

L. Veress, L. Lovas, L. Radnai
Agricultural College, Kaposvár
Department of Animal Husbandry
7400 Kaposvár, Dénesmajor 10.

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APPLICABILITY OF MORPHOLOGICAL MAIZE MUTANTS IN PLANT BREEDING*

At the University of Agriculture, Debrecen, the effect of various physical and chemical mutagens have been studied for more than 10 years, mainly on maize. Over the years maize seeds were treated at the Nuclear Research Institute, Debrecen, the Department of Radiology of the Medical University, Debrecen and the Radiation Garden of the University of Agriculture, Gödöllő. Subsequently irradiations were also carried out in the Central Research Institute for Physics and in the Experimental Nuclear Reactor of the Technical University, Budapest.

The treatments yielded a mutant population containing a large variety of forms. From the point of view of selection, almost all the available mutants had some useful properties. This indicates that, by using various mutagens, the variability of plant forms can be increased in order to provide basic breeding stock. The induction of certain morphological characteristics may give especially important results.

Without going into details, I should like to discuss the properties of some of our new maize mutants.

Multi-eared mutants

An increase in the ear number per stalk leads to an increase in the yield and yield component. The production of this type of basic stock may be of considerable importance in plant breeding (Figs 1—7).

There are two methods for producing plants with several ears. Firstly, by selecting mutants which develop 3, 4 or more ears on the multilateral nodes bearing the ears (Fig. 1). Secondly, by using types in which a spike-like ear formation with 4—5 ears develops on the generative peduncles formed from the multilinear nodes (Figs 2, 3 and 4). Both types are suitable for producing multi-eared hybrid maize. By using various methods of plant breeding, these morphological mutants can be used to produce even more productive maize hybrids. Another mutant, which seems to be important, forms ears directly below the tassel (Figs 5, 6 and 7). By producing this type of hybrid, the number of stalks per hectare can be increased as the shielding effect is not manifested and cannot therefore inhibit the development of the ears.

Corn-grass mutants with narrow leaves

Corn-grass type varieties with narrow leaves can also be found in our mutant population. These have a thin stalk and narrow leaves and have a tendency to grow into a bush. On hybridizing with normal inbred maize, a high degree of heterosis is obtained. These types can be utilized in the development of superior strains of silage maize. From types with a larger degree of tillering capacity maize hybrids suitable for grazing can also be produced (Fig. 8).

Female unisexual mutant

Of the morphological mutants, the so-called female unisexual maize mutants is very important and may play an important role in the production of hybrid seed (Figs 9 and 10). The first such mutant was discovered last year. Subsequently, it was used in top crosses in order to find parent partners for maintaining and terminating unisexuality. If such types are found, they may form a new basis for the production of hybrid maize seed.

* Lecture held at the meeting of the ESNA (European Society of Nuclear Methods in Agriculture) in Uppsala, Sweden, from 29th August to 2nd September 1977.

Fig. 1. Multi-eared maize mutant. The ears are one above the other on the main stalk. 4 or 5 ears may develop on each shoot

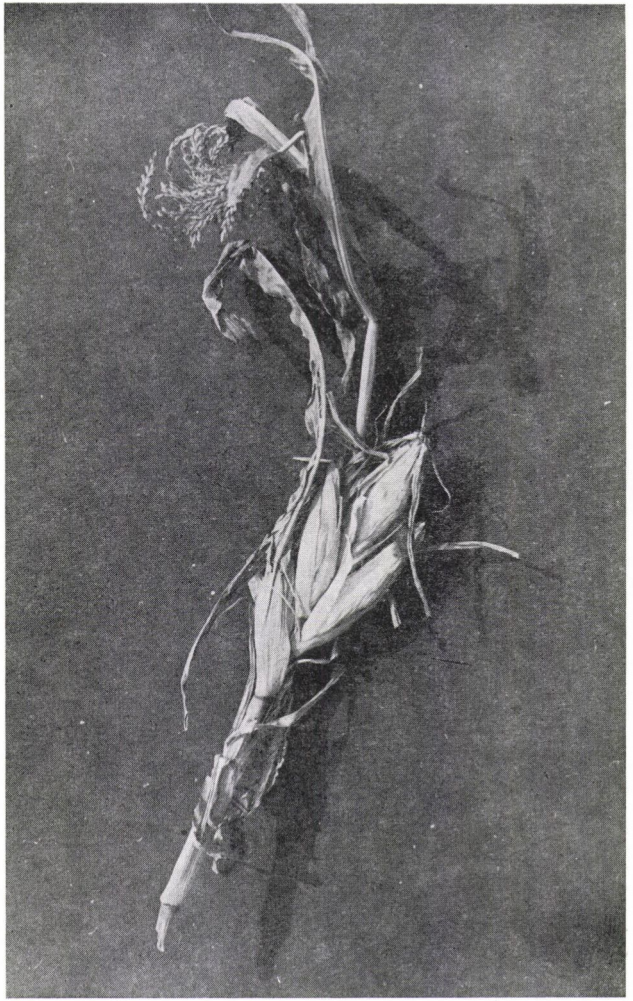

Fig. 2. Multi-eared maize mutant. One generative shoot develops from the main stalk and this produces 4 or 5 ears. Since the ears develop very close together the whole ear formation resembles a spikelet

Fig. 3. Multi-eared maize mutant. Several forms of ear formation can be seen in the picture. The ears are placed like spikelets and individually, or only like spikelets on the generative stalk part developing from the main shoot. 3—6 ears may be found on each stalk

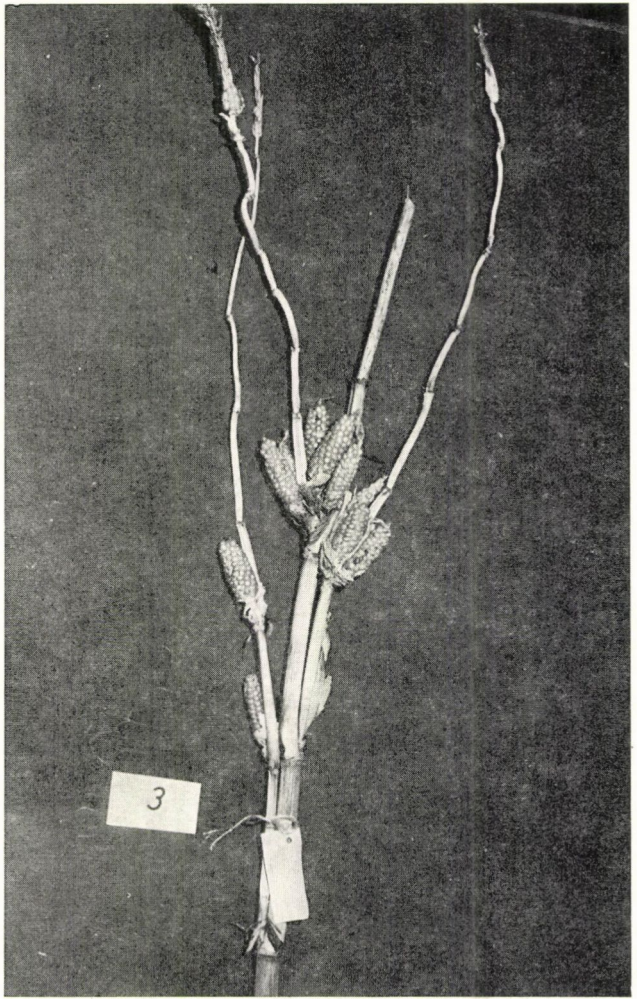

Fig. 4. Multi-eared maize mutant. The ears are arranged in a bunch. This is a branching type, where ears can also be found on the branches, and both male and female flowers occur at the ends of the branches

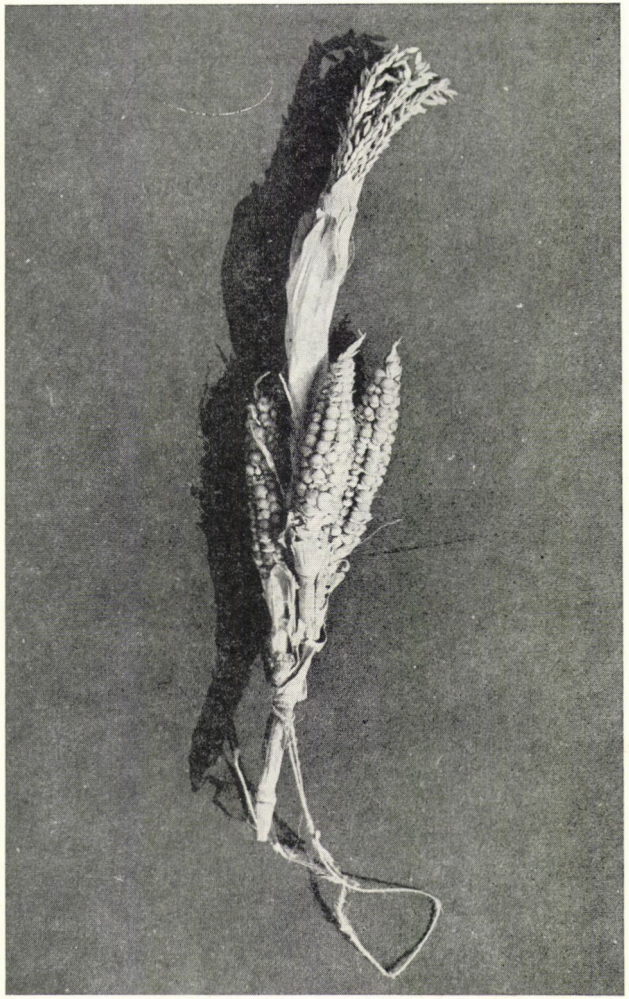

Fig. 5. Maize mutant with ear formation below the tassel. The 3 ears almost completely surround the tassel

Fig. 6. Maize mutant with ears developing immediately below the tassel. Below the spicate, somewhat under-developed tassel there are generally 12—14 ears, placed concentrically. At the base of each ear a narrow leaf is formed

Fig. 7. Maize mutant, the 4 ears of which are found directly at the base of the tassel, all on one side

Fig. 8. Mutant plants of the narrow-leaved, strongly tillering "corn-grass" type. On the left of the picture a tillering, narrow-leaved, dwarf plant can be seen, and on the right a strongly tillering, tall plant with narrow, erect leaves

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Fig. 9. Bushy, unisexual, female type maize mutant with narrow, sedge-like leaves

Fig. 10. Unisexual, female type of branching maize mutant with erect leaves

Only some of the several hundreds of morphological maize mutants are presented here, indicating the importance of mutation in terms of providing basic plant breeding material. By the appropriate use of various mutagens, a genetically diversified basic stock was produced which was used for plant breeding, therefore making possible the production of highly productive plant species.

K. PÁSZTOR
Plant Growing Department,
University of Agriculture,
4001 Debrecen,
Böszörményi út 138.

COMPARATIVE STUDY ON THE ALKALOID PRODUCTION OF SOLANUM DULCAMARA CHEMOTAXA DURING THE VEGETATION PERIOD*

Solanum dulcamara L. belongs to the few European native species of the genus *Solanum*. In the plant alkaloids with a spirosolan skeleton, namely tomatidenol, solasodine and soladulcidine are found in various proportions. These alkaloids can be transformed to a varying extent into steroid-hormone intermediaries. This explains the fact that many authors in a number of European countries have carried out intensive studies on the plant. The large number of analyses is partly due to the interesting finding that the alkaloid composition of the plants varies with their geographical origin (BOLL—ANDERSEN 1962, BOGNÁR—MAKLEIT 1965, MAKLEIT *et al.* 1967, MÁTHÉ—MÁTHÉ Jr. 1972, 1973, SANDER 1963, SCHREIBER—RÖNSCH 1963, WILLUHN 1966, VO HONG NGA *et al.* 1976). The differences characteristic of the vegetative organs can be brought into connection with parameters referring to the active agent production of the plant. These parameters as well as other questions related with the variability of the active agent of the plant have been dealt with in other papers by the authors (MÁTHÉ Jr.—MÁTHÉ 1978a, 1978b).

In this lecture reference will be made, among others things, to the relationship between the alkaloid composition in the vegetative organs and the alkaloid production. For example, a production analysis made at a given time of taxa containing tomatidenol and soladulcidine and grown under identical conditions has revealed that in Hungary plants containing soladulcidine produce a larger phytomass, while, owing to the higher alkaloid content of the organs, the alkaloid production was found to be more favourable in the taxon containing tomatidenol. Since these results were obtained on a single occasion with some 30 plants collected from various sites, it seemed reasonable to extend the comparative study of these taxa to an almost full vegetation period and a more homogeneous plant material, in order to control the correctness of the correlation established. The comparison was made between plants containing no soladulcidine, and those containing soladulcidine in the aboveground organs.

On 30th April 1975 a stand was produced by vegetative reproduction, with cuttings from a selected mother plant containing tomatidenol as the main alkaloid (and only a little solasodine). Another population was produced by cloning a specimen grown from the seed of the same plant. Since the latter was established by cloning a plant grown from the seed obtained from fruit produced in Hungary on a tomatidenol-containing plant, it contained

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soladulcidine as well as tomatidenol. Under Hungarian conditions the plants mostly contain soladulcidine aglycon (MÁTHÉ—MÁTHÉ Jr. 1972), and at our experimental site it is possible that crossing took place.

From each of the two populations thus established 10 plants were collected every two weeks; the aboveground organs were sorted, dried at 70° C and weighed; the data obtained were averaged and included in the tables as phytomass values.

After extraction with low concentration acid and hydrolysis with hydrochloric acid, the alkaloid contents of the individual organs were determined titrimetrically in a chloroform-containing medium using p-toluene sulphonic acid in the presence of dimethyl yellow indicator. The values obtained were expressed as the solasodine percentage of the dry drug (MÁTHÉ Jr. 1970).

The alkaloid composition, i.e. the presence or absence of soladulcidine, was checked by means of chromatography using chloroform—methanol (19 + 1) as the running medium, on a silica gel-G plate. The spots were made visible with antimony trichloride in chloroform as the reagent. ROZUMEK's (1969) chromatographic method was also used to demonstrate the presence of soladulcidine.

Some data on the weather conditions of the period examined together with the 1976 data are shown in Table 1; the table was prepared using data from the Gödöllő and Vác meteorological stations. The data indicate how much the vegetation period of 1975 differed from that ln 1976, a period which corresponded more closely to an average summer season in Hungary.

Table 1

Trends in some meteorological factors during the vegetation period at Vácrátót (1975, 1976)

	Months					
	April	May	June	July	August	September
1975						
Number of hours of sunshine	188	219	223	284	210	224
Average daily mean temperature, °C	10.2	17.1	18.3	20.4	19.5	17.4
Precipitation, mm	35	46	79	133	69	51
1976						
Number of hours of sunshine	202	250	316	313	236	120
Average daily mean temperature, °C	11.1	15.1	18.5	21.4	17.6	14.5
Precipitation, mm	67	25	31	58	17	130

Data concerning the whole aboveground phytomass as well as the percentage distribution between the organs for the two taxa are compared in Table 2. The first collection was carried out on 2nd July 1975.

In the first half of the vegetation period, up to the 9th week (21st August) there was no significant difference in production values between the two taxa. This was also expressed in the fact that the average trend for the full phytomass during the first nine weeks showed

Table 2

Percentage changes in the aboveground phytomasses and organs of Solanum dulcamara L. chemotaxa during the vegetation period (1975)

Week	Dry phytomass (g)		Percentage distribution of phytomass by organs									
			Leaf		Stem		Flower		Fruit			
									green		ripe	
	1	2	1	2	1	2	1	2	1	2	1	2
1	1.9	5.3	67.5	67.1	31.3	29.7	1.2	0.1		3.1		
3	13.1	13.4	58.9	60.4	35.5	32.6	3.6	2.1	2.0	4.9		
5	17.4	18.2	52.5	51.6	39.2	40.4	4.9	4.2	3.4	3.9		
7	46.3	29.4	44.3	42.5	44.2	42.7	2.6	1.9	8.9	12.9		
9	100.4	70.6	36.8	35.1	41.4	43.7	2.1	2.4	19.7	18.8		
11	96.6	105.0	32.3	26.6	45.0	46.8	3.6	1.0	18.6	20.6	0.6	5.0
13	148.6	173.3	28.1	19.1	50.8	52.6	2.0	0.1	16.4	12.7	2.7	15.5
15	173.8	195.2	25.7	12.9	60.1	65.7	0.9	0.0	10.2	4.55	3.0	16.8
17	193.6	218.2	25.9	10.8	57.9	71.0	0.1	0.0	8.5	3.5	7.5	14.7

1 = Soladulcidine-free taxon.
2 = Soladulcidine-containing taxon.

no significant difference between the two taxa either at the P = 5% or at the P = 10% level. (The average difference during that period was $\Delta X = 7.4 \pm 5.8$. On the basis of the available data the soladulcidine-free taxon had the larger average production, even though this could not be mathematically proved.)

Table 3

Average difference in phytomass between Solanum dulcamara L. taxa containing and lacking soladulcidine in the period of fruit ripening (11th—17th week)

Plant parts	Difference in dry phytomass (g)		Difference in percentage proportion of organs	
	$\overline{\Delta X}$	SD	$\overline{\Delta X}$	SD
Leaf	11.3	±3.4	10.6	±1.8
Stem	—22.8	±6.7	—5.6	±2.7
Flower	1.7	±0.5	1.5	±0.4
Fruit				
Green	4.1*	±5.3	3.1	±2.5
Ripe	—15.6	±4.2	—9.5	±1.9
Aboveground total	—20.4	±3.7		

* At the P = 5% level of significance there is no difference between the two taxa.
The negative sign means that the higher values were obtained from the taxon containing soladulcidine.

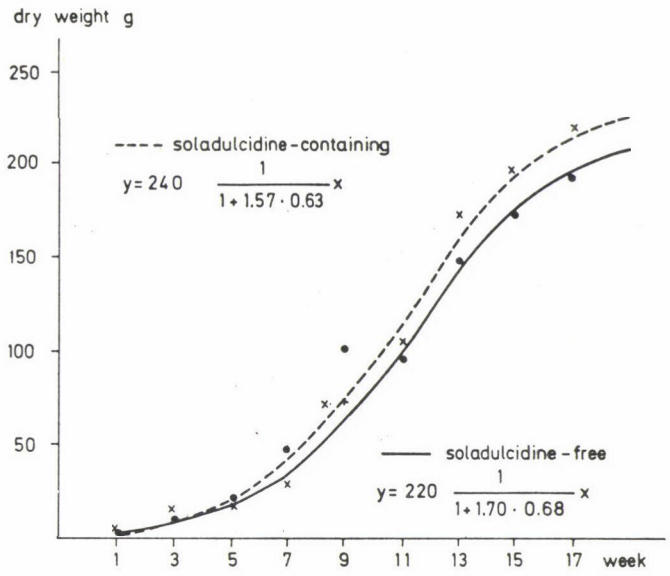

Fig. 1. Trends in the aboveground phytomasses of *Solanum dulcamara* L. taxa (1975)

For this reason our examinations were concentrated on the period from the 11th to the 17th week (up to 15th October), when there were already ripe fruits on the plants, and the two taxa showed a mathematically demonstrable difference. On the whole the taxon that also contains soladulcidine produced more than the soladulcidine-free taxon (Table 3).

When considering the volume of stems and ripe fruits within the whole phytomass it was found that the taxon containing soladulcidine gave the higher values, so much so that these organs were decisive for the difference in the whole phytomass too, in spite of the fact that the leaf production was higher in plants not containing soladulcidine. The differences shown in the table, except for those in the weight of the green fruit, were verifiable at the $P = 5\%$ level of significance.

Changes in the full phytomass during the whole period are shown in Fig. 1; a logistic curve was fitted to the values obtained, the reason for which is discussed in another publication (MÁTHÉ Jr.—MÁTHÉ 1978c).

The percentage distribution of the aboveground phytomass among the different organs, again from the eleventh week on, is also shown in Table 3. The difference between the two taxa is significant at the $P = 5\%$ level for each organ, so it can be unequivocally established that in the fruit ripening period the share of the stem and the ripe fruit in the total aboveground production of the plant is higher in strains containing soladulcidine than in those not containing this alkaloid.

A characteristic difference was found between the two taxa in the percentage alkaloid content in the organs. Fig. 2 shows the changes in the alkaloid contents of green fruit, leaf and stem. On the basis of the figure it can be established that the organs of the taxon containing tomatidenol produce more alkaloid than the corresponding organs of the plant material which also contains soladulcidine.

The alkaloid contents in the individual organs over an average of the vegetation period are shown in Table 4, together with the standard deviations of the data. These latter values

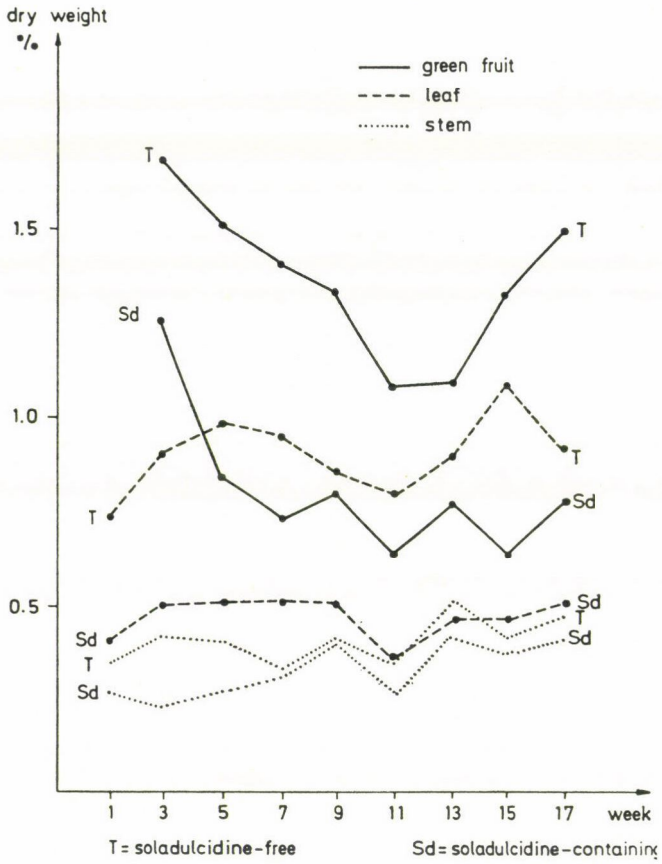

Fig. 2. Alkaloid contents (dry weight %) in the organs of *Solanum dulcamara* L. taxa with tomatidenol as main aglycon, containing or lacking soladulcidine (1975)

point to a fluctuation in the alkaloid contents during the vegetation period. As expected on the basis of earlier publications, this table also shows that in the strain lacking in soladulcidine the alkaloid content was higher in each organ than in the strain containing soladulcidine. The greatest fluctuation was found in the generative organs, which is in good agreement with the phenomenon of alkaloid change related with the development of the fruit, as discussed in earlier papers by the authors (MÁTHÉ Jr.—MÁTHÉ 1978a, 1978b).

The trend of the aboveground alkaloid production is seen in Fig. 3. According to the figure, up to the end of the vegetation period the alkaloid content follows a practically logistic curve in both taxa. There is an essential difference, however, in the parameters indicating the saturation is 1.3 g for the taxon containing tomatidenol, and 1 g for that containing soladulcidine.

Differences between the two taxa with respect to the alkaloid contents of the individual organs at the time of fruit ripening are shown in Table 5. The table reveals that the tomatidenol-containing taxon produces more alkaloid in all the organs; the differences are the greatest for the leaf and green fruit.

Table 4

Average alkaloid content (dry weight %) in the organs of Solanum dulcamara L. taxa, and its fluctuation (standard deviation of data) during the vegetation period (1975)

Organs	Taxa			
	Soladulcidine-free		Soladulcidine-containing	
	$\bar{x}$	s	$\bar{x}$	s
Leaf	0.90	±0.11	0.46	±0.07
Stem	0.41	±0.09	0.33	±0.09
Flower*	0.64	±0.30	0.57	±0.09
Fruit				
Green	1.29	±0.40	0.76	±0.16
Ripe	0.46	±0.15	0.21	±0.05

* At the P = 5% level of significance there is no difference between the taxa.

Table 5

Difference in alkaloid production between Solanum dulcamara L. chemotaxa in favour of the soladulcidine-free taxon in the period of fruit ripening (g/plant)

Organs	Alkaloid (g)	
	$\overline{\Delta X}$	SD
Leaf	0.227	±0.034
Stem	0.035	±0.024
Flower*	0.013	±0.003
Fruit		
Green	0.137	±0.059
Ripe	0.040	±0.016
Aboveground total	0.303	±0.078

* At the P = 5% level of significance there is no difference between the taxa.

On the basis of the results introduced above the following conclusions can be made:

The difference between the two populations became conspicuous in the second half of the vegetation period, after the ripe fruit had appeared. As regards the production of the individual plant organs, it was found that in the taxa containing soladulcidine the surplus that practically decided the total phytomass production was supplied by the stem and the ripe fruit. It was, however, remarkable that even in the period of fruit ripening the leaf phytomass was considerably larger in the soladulcidine-free population than in the taxon containing soladulcidine.

The differences are more or less the same when we compare the taxa for the percentage of the individual plant parts. These results suggest that in the second half of the vegetation

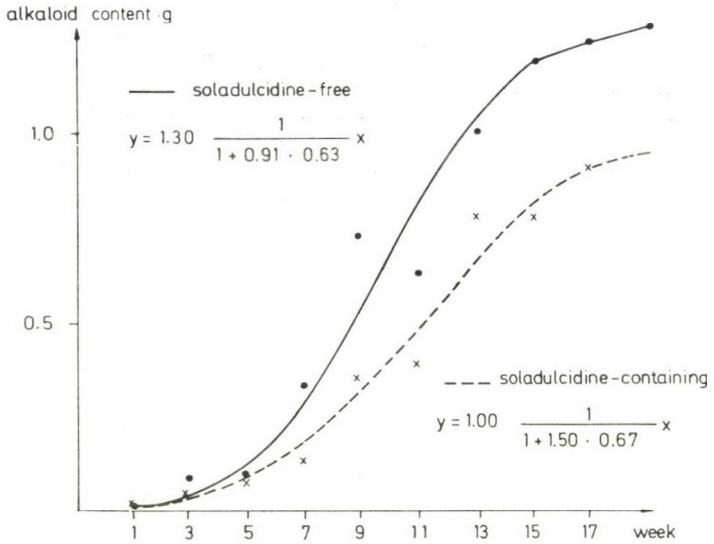

Fig. 3. Changes in the alkaloid production of *Solanum dulcamara* L. taxa (1975)

period the soladulcidine-containing population shows a more advanced stage of development than that containing tomatidenol as the main aglycon. In 1975 when the distribution of precipitation, the average temperature and the number of hours of sunshine substantially deviated from those (e.g. in 1976) more appropriate to the continental character of Hungary, the tomatidenol-containing stand remained in a more vegetative stage throughout the growth season than the population containing soladulcidine.

These results agree with the observations of GAUHL (1968) who compared ecotypes obtained from shaded and sunny habitats (Western and Eastern Europe) for photosynthetic activity in a phytotron experiment (leaving the alkaloid composition out of consideration). He found that when exposed to intensive illumination the ecotype originating from a sunny habitat showed increased production (protein, carbohydrate production, CO_2 uptake), while the same increase was not observed in the photosynthetic activity of the ecotype from a shaded habitat. In our experiment the tomatidenol-containing taxon obtained from Western Europe corresponded to the shady ecotype, while our plant containing soladulcidine was more or less similar to the soladulcidine-containing taxon commonly found in Hungary. Our data reveal that as long as the number of hours of sunshine was relatively low (first half of the vegetation period), the difference in production between the two taxa was not significant. In the period of fruit ripening, on the other hand, the soladulcidine-containing population made better use of the increased illumination, as manifested by its phenologically more advanced stage. It must be emphasized, however, that the results obtained in 1975 only allow cautious generalizations.

Differences in alkaloid content between the two taxa were to be expected on the basis of earlier investigations (MÁTHÉ—MÁTHÉ Jr. 1973). Since, however, the two populations were of the same origin (having been obtained by vegetative and generative propagation on the same plant), the results are even more clear-cut than those of the earlier experiment. It has been established once again that each organ of the soladulcidine-free population has a higher alkaloid content (dry weight %) than the corresponding organ of the stand containing soladulcidine. (It is only in the flower that this difference is negligible.)

Among the organs, changes in the alkaloid content of the green fruit are not worthy of attention, as they are considerably influenced by the stage of development and the ripeness of the fruit. Our earlier investigations raised the suspicion that apart from the developmental stage the alkaloid composition characteristic of the vegetative organs was also related with the alkaloid content of the fruit. Since the alkaloid content of the fruit was higher during the whole vegetation period in the soladulcidine-free population, the conclusion can safely be drawn that it is not only the developmental stage of the fruit but also the alkaloid composition (in the present case the presence or absence of soladulcidine) in the vegetative organs that influences, or rather, has something to do with the alkaloid level of the fruit.

It should be mentioned here that, as seen in Fig. 2, towards the end of the vegetation period the alkaloid content was found to increase in the fruit compared to the earlier phases of the period examined. This can probably be explained by the cooler, rainier weather in August compared to other years, which was favourable for the development of new shoots where later new flowers and new fruits were formed.

To sum up, it can be seen that while the phytomass production was larger in the population containing soladulcidine, the alkaloid content (dry weight %) was more favourable in the soladulcidine-free stand; that is, from the point of view of alkaloid production the two factors considered showed opposite tendencies. Since the difference in alkaloid content (dry weight %) between the two stands was relatively higher than the difference between their phytomasses, and the leaf : fruit ratio and the quantity of leaf and fruit per plant were also more favourable in the tomatidenol-containing (soladulcidine-free) population, from the point of view of alkaloid production the population containing tomatidenol was found to be unequivocally better, as is expressed in Fig. 3, which illustrates the alkaloid production per plant, and in Table 5.

I. MÁTHÉ Jr., I. MÁTHÉ
Research Institute for Botany
of the Hungarian Academy of Sciences
2163 Vácrátót

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 28 (3—4), pp. 467—470 (1979)

RECENSIONES

Bayerische Landesanstalt für Tierzucht Grub, Jahresbericht 1976, Band 16.

I. The first chapter of the year-book describes the organization of the institute. The institute consists of the following departments: Cattle Breeding Department, Pig and Sheep Breeding Department, Small Animal Breeding Department, Feeding and Feed Preservation Department, Construction and Technology Department.

II. The major subjects investigated at the institute in 1976 were:

Breeding. Examination of correlations between individual performance, performance of members of the same progeny, and progeny performance. Investigations on the utilization of the heterosis effect. Study of phenotype and genotype parameters. Improvement of methods for assessing the breeding value. Examination of correlations between fattening performance and slaughter quality.

Production technology. Examination of question involved in keeping cows. Milking technology studies. Testing of various types of automatic feeders in pig fattening. Effect of UV irradiation on the results of pig fattening. Early detection of stress sensitivity in live hogs. Effect of early weaning of lambs on the productivity of the ewes, on the period between lambings and on the health of the ewes. Effect of rearing methods on the performance of lambs.

Husbandry technology and applied ethology. Comparison of various cattle husbandry technologies. Keeping milking cows in free pens. Keeping sows in a bound system. Systems of liquid manure treatment. Chemical reduction of stink in pig farms.

Feeding. Effect of the preserving technology on feed uptake. Different intensities of heifer rearing. Studies on various methods of straw extraction. Experiments with milk substitutes for calves. Effects of various additives in pig fattening.

Fodder preservation. Examination of the effect of various silage additives. Effect of the technical conditions of ensilation on losses and digestibility. Ensilation of grain maize

and other grain crops. Examination of the costs of hot air drying.

Veterinary hygiene. Tests of antibiotic residues in milk, blood and urine. Milk hygiene tests. Effect of vitamin A and carotene feeding on fertility in cows.

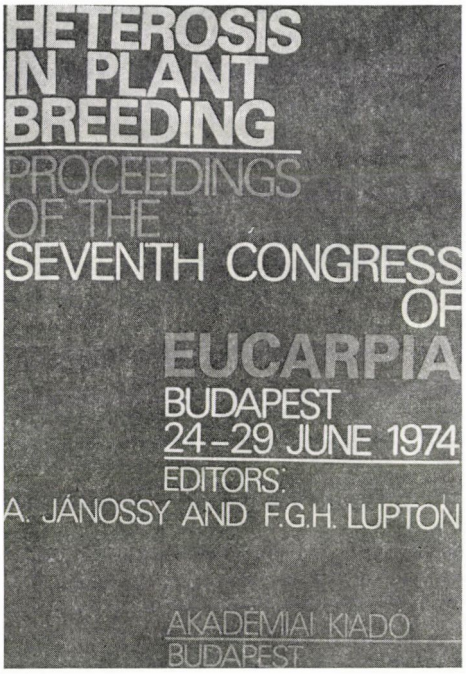
III. Educational and advisory activities at the institute. The subjects of courses held at the institute, the number of participants, and the scientific programmes organized at the institute are listed in this chapter.

IV. This chapter contains the numerical results of performance studies on cattle, pigs, sheep and rabbits.

V. The major literary works published by the members of the institute are as follows: H. Bogner *et al.:* Animal breeding, 2nd ed.; H. Alps *et al.:* Preliminary results obtained at the progeny testing stations in individual performance and progeny tests on bulls; H. Blendl *et al.:* Contribution to objective meat qualification in pigs; W. Peschke and G. Averdunk: Results of breeding value assessment in young sows; G. Burgstaller and W. Knirsch: The use of barley varieties with different protein contents in pig fattening; G. Burgstaller *et al.:* Pig fattening experiments with Pesonnitrovin; G. Burgstaller *et al.:* The use of pulverized and sugar-reduced whey in calf fattening; F. Gross *et al.:* Chemical and microbiological studies on ensiling pre-dried red clover supplied with N-fertilizer; R. Ivanovski: Performances of sows suckling for three weeks and fed at different intensities; H. Blendl: Chemicals in fighting stink; H. Blendl: Liquid manure and environment pollution; W. Csedek *et al.:* Levels of antibiotics in urine and tissues in calves; J. Coropp *et al.:* Effect of growth stimulants on fattening and slaughter outputs in calves; W. Krenzer and W. Hollwich: Mercury content in fish originating from South-German waters, and its food hygiene aspects; P. Senger: Effect of early weaning of piglets on some blood parameters as well as on fattening performance and slaughter value.

VI. The year-book is completed with a list of 205 further literary works published by the members of the institute in 1976.

J. SCHMIDT



JÁNOSSY, A., LUPTON, F. G. H.: *Heterosis in Plant Breeding.* Proceedings of the Seventh Congress of EUCARPIA, Budapest, 24—29th June 1974. The European Association for Research in Plant Breeding. Akadémiai Kiadó, Budapest, 1976. pp. 366.

This 366-page book contains the material of the seventh congress, five sections being devoted to lectures delivered by the members followed by those held by non-members, in the form of brief abstracts. The congress was organized in Budapest at the Horticultural University between 24th and 29th June 1974.

The Congress was opened by the chairman, Dr. F. G. H. Lupton, who pointed out that the phenomenon of heterosis, which ensures increased yields, is already widely used in practice by breeders all over the world and that further results may be expected from a study of the theoretical bases. A. Jánossy, academician, the deputy chairman of the Congress, greeted the participants on behalf of Hungary, the host country.

Prof. A. Jánossy died before the volume was published; a necrology giving details of

his scientific work is presented in the introduction of the book.

Section 1, with Prof. G. Wricke and I. Bócsa as chairmen, discusses the genetic principles of heterosis, giving information on the following subjects: Genetic and evolutionary aspects of heterosis in relation to allele, semiallele and plasmic and/or parental interactions, with special regard to disomic polyploidy. Population genetics was treated through a mathematical description of dynamic changes in the hybrid populations. Of the theoretical problems of inbreeding, spontaneous and induced mutation, productivity and pathological resistance were dealt with from a genetic point of view. The complementation of allodiploidy in wheat and the evolution of the homoeologic molecular systems of enzyme subunits are interpreted by heterosis interactions between the genomes. In the case of tomato the method of selecting for heterosis between male sterile parent plants and those without stamens was described. It was established that in Fragaria hybrids heterosis was manifested in an increased vegetative character.

Section 2 deals with the physiology of heterosis, under the chairmanship of Mr. A. Lein and Mr. Á. Kiss. Lectures were delivered on the following subjects: Manifestation and quantitative data of dry matter, leaf size and photosynthetic activity in heterosis, primarily in wheat. The biological and biomechanical aspects of heterosis were evaluated in relation to photosynthesis, cell respiration, fermentation activity, mitochondrial activity, the relative quantities of isozymes, the levels of endogenous regulators (hormone-like bioactive compounds), etc. In the heterosis of wheat the relative ratios of chlorophyll components, the photosynthetic activity and the intensity of photorespiration, at high temperatures and in connection with the combining ability of the varieties, were discussed. The fertility of the restoration male sterile line containing 21 Rf genes in the cytoplasm of *Triticum timopheevi* was another subject in this section. The results of a comparative study on heterosis by determining the mitochondrial complementation and the cytochrome c oxidase activity under laboratory and greenhouse conditions were evaluated; a correlation coefficient (r) of 0.61 or lower indicated a low grain yield.

Section 3, with Mr. J. Sneep and Prof. A. Bálint as chairmen, summarizes the methods of heterosis breeding. The following subjects were treated: Sensitivity to the environment exhibited by fertility restoring genes in wheat, as proved by the increase of male fertility in autumn sowing. Selection for self-fertility in *Begonia*, *Tagetes*, etc. through heterosis with a simultaneous increase in adaptive polymorphism, ecological tolerance and chromosomal heterozygosis. Transmission of the restoration ability of cytoplasmic male sterility in sterile *Triticum timopheevi*, and its utilization in hybrid wheat breeding. Experimental evaluation of heterosis in winter wheat on the basis of 23 F_1 crossings in microplots between 1971 and 1973, with a view to characterizing the combining ability of the parental generations. Productivity of the F_1 hybrids of fodder grasses with investigations into the incompatibility systems, in correlation with the frequency of two loci. Assessment of the heterosis level in the F_1 and F_2 generations of wheat and maize on the basis of the parental characters, by evaluating the biometric interaction, the most important components influencing the yield and the raw crop.

Section 4 discusses heterosis in cross-pollinated species under the chairmanship of Prof. G. Röbbelen and Prof. E. Kurnik. The subjects dealt with were: Comparison of hybrid populations originating from inbred lines with lines obtained through the selection of recurrent hybrids by evaluating the heterozygosis and establishing the frequency of covered dominance and overdominance, in connection with polyploidy levels and epistatic effects. Genetic erosion of F_1 hybrids in carrot, onion and other vegetables, manifestation of productivity, earliness, pathological resistance and market value in the heterosis effect. Manifestation of cold tolerance in the heterosis of Opaque-2 and its analogues in three way cross and double cross hybrids in connection with the occurrence of

the opaque gene (o_2) in recessive homozygotes, with the manifestation of cold tolerance and germination at low temperatures. Possibilities of using heterosis in the case of three way cross hybrid lucerne on a 15 F_2 male sterile line by utilizing the cytoplasmic male sterile system. Evaluation of the combining ability of new lines after selection on the basis of heterosis, and the production of F_1 hybrids in vegetable marrow, by applying Ethephon. Production biological evaluation of intervarietal and inbred hybrids of grasses *(Lolium multiflorum)* on the basis of the heterosis of cyclic and diallele crosses.

The subject of Section 5 is heterosis in self-fertile species. Chairmen: Prof. H. Hänsel and Mr. S. Rajki. The topics in this section were: Heterosis of barley hybrids in the F_1 generation in investigations made between 1967 and 1973 on the genotype and environmental effect, and characterization of the self-fertile, high productivity line segregated. Characterization of the heterosis of hybrid wheat as tested with the method of mitochondrial complementation, and the evaluation of the dimensions of a hybrid population and the determination of the extent of fertilization caused by pollen shedding, using coloured marker genes. Wheat crossing in the case of 61 intergeneric hybrids with examinations of the heterosis and combining ability, in order to evaluate the yield components with restored fertility after the chlorophyll level had been influenced by hybridization in one positive and two negative cases. Evaluation of the heterosis of soft winter wheats in 92 hybrid combinations on the basis of 430 cases of preliminary crossing between 1967 and 1973 on non-chernozem type soils in the Soviet Union. Hybrid wheat research at Martonvásár with the qualification of the heterosis of winter wheat on the basis of grain yield and plant weight, with restored fertility. Interactions of heterosis and hereditary factors of wheat in the weight of the plants, the number and weight of grains per ear and the protein content per grain. Evaluation of heterosis in tomato from the point of view of earliness, high yield, pathological resistance to virus and fungal infections, etc. Genetic study on four rice hybrids in the F_1 and F_2 generations with an evaluation of heterozygosis and determination of the correlation between plant height, length of panicle and number and weight of grains.

Abstracts of papers submitted to the Congress. Genetic system of partially allogamous broad bean in diallele crosses, for the determination of the course of transmission. Evaluation of hybrid vigour in the synthetic variety of *Dactylis* with the determination of the frequency of tetragenic loci and the qualification of the combining ability. Correlation between fertility and the restoration of pollen fertility in some flowering wheat varieties, with the determination of the pleiotropic effect of Rf genes using the backcrossing of progenies. Manifestation of heterosis and combining ability in diallele wheat crosses with the evaluation of yield components (number of grains per ear, grain weight and thousand-grain-weight). Study of the effect of heterosis in some intervarietal tabacco hybrids, with the evaluation of the F_2 generation between 1968 and 1972. Quantitative heterosis differences in the species Arabidopsis thaliana.

The volume is completed by news and reports of the Eucarpia organization, a list of members, a list of the participants at the Seventh Congress, and an account of activities between 1971 and 1974.

B. I. Pozsár

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AUCTORES

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MTA Mezőgazdasági Kutatóintézete,
2462 Martonvásár,
Hungary

DIBUZ E.
KE Növénytani Tanszék,
1118 Budapest,
Ménesi út 44.
Hungary

FARKAS E.
KE Kémiai Tanszék,
1114 Budapest,
Villányi út 29—31.
Hungary

FAZEKAS S.
SOTE II. sz. Kémiai-Biokémiai Intézete,
1088 Budapest,
Puskin u. 9.
Hungary

FEHÉR D.
KE Kémiai Tanszék,
1114 Budapest,
Villányi út 29—31.
Hungary

FRENYÓ V.
ELTE Növényélettani Tanszék,
1088 Budapest,
Múzeum krt. 4/a.
Hungary

GRACZA P.
Gyümölcs- és Dísznövénytermesztési Kutatóintézet,
1223 Budapest,
Park u. 2.
Hungary

KISS A. S.
BVK Agrokémiai Osztály,
3700 Kazincbarcika,
Hungary

KORBULY J.
Bányászati Kutatóintézet,
1037 Budapest,
Mikoviny u. 2—4.
Hungary

KOVÁCS G. D.
GYDKI Kutató Állomása,
2701 Cegléd,
Szolnoki út 52.
Hungary

KOZMA P.
KE Szőlőtermesztési Tanszék,
1114 Budapest,
Villányi út 35—43.
Hungary

KÜKEDI E.
MTA Mezőgazdasági Kutatóintézete,
2462 Martonvásár,
Hungary

LAKATOS B.
MTA Központi Kémiai Kutatóintézete,
1525 Budapest,
Pusztaszeri út 59—67.
Hungary

LELLEY J.
6721 Szeged,
Vidra u. 1. III. e. 1.
Hungary

LOVAS L.
MF Állattenyésztési Tanszék,
7401 Kaposvár,
Dénesmajor 2.
Hungary

MÁTHÉ I.
MTA Botanikai Kutatóintézete,
2163 Vácrátót,
Alkotmány u. 2—4.
Hungary

MÁTHÉ I. Jr.
MTA Botanikai Kutatóintézete,

2163 Vácrátót,
Alkotmány u. 2—4.
Hungary

MEISEL J.
MTA Központi Kémiai Kutatóintézete,
1525 Budapest,
Pusztaszeri út 59—67.
Hungary

NÉMETH J.
Gabonatermesztési Kutatóintézet,
6701 Szeged,
Alsókikötősor 9.
Hungary

ÓVÁRY I.
SOTE II. sz. Psychiatriai Klinika,
1083 Budapest,
Balassa u. 6.
Hungary

PAIS I.
KE Kémiai Tanszék,
1114 Budapest,
Villányi út 29—31.
Hungary

PÁSZTOR K.
DATE Növénytermesztési Tanszék,
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Hungary

PINTÉR L.
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Hungary

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Hungary

POZSÁR B. I.
MTA Izotóp Intézete,
1525 Budapest,
Konkoly Thege út
Hungary

RADNAI L.
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Dénesmajor 2.
Hungary

RAJKI E.
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2462 Martonvásár,
Hungary

SCHMIDT J.
KATE Állattenyésztéstani Tanszék,
9201 Mosonmagyaróvár,
Vár 4.
Hungary

SIMON I.
GYDKI Kutató Állomása,
9431 Fertőd,
Hungary

SINGH K.
Department of Soil Science,
Govind Ballabh Pant University of Agriculture and Technology,
Pantnagar, District Nainital,
Uttar Pradesh,
India

SIPOS S.
JATE Kolloid Kémiai Tanszék,
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Aradi Vértanúk tere 1.
Hungary

SUTKA J.
MTA Mezőgazdasági Kutatóintézete,
2462 Martonvásár,
Hungary

SZABÓ Cs.
GYDKI Kutató Állomása,
9431 Fertőd
Hungary

SZABÓ M. K.
KE Szőlőtermesztési Tanszék,
1114 Budapest,
Villányi út 35—43.
Hungary

SZARVAS T.
MTA Izotóp Intézete,
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SZÉKESSY-HERMANN V.
SOTE II. sz. Kémiai-Biokémiai Intézete,
1088 Budapest,
Puskin u. 9.
Hungary

TERPÓ A.
KE Növénytani Tanszék,
1118 Budapest,
Ménesi út 44.
Hungary

UDVARDY J.
MTA, SzBK Növényélettani Intézete,
6701 Szeged,
Odesszai krt. 62.
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VERESS L.
MF Állattenyésztési Tanszék,
7401 Kaposvár,
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